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LETTERMAN ARMY INSTITUTE OF RESEARCH ANNUAL PROGRESS REPORT, FY 1973

William A. Akers

Letterman Army Institute of Research
San Francisco, California

30 June 1973

AD_____

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO
CALIFORNIA 94129

REPORT NO. 17

ANNUAL PROGRESS REPORT, FY 1973

RCS SGRD-288(R1)

30 June 1973

UNITED STATES ARMY

MEDICAL RESEARCH AND DEVELOPMENT COMMAND

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In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care,' as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of of Laboratory Animal Resources, National Academy of Sciences - National Research Council.

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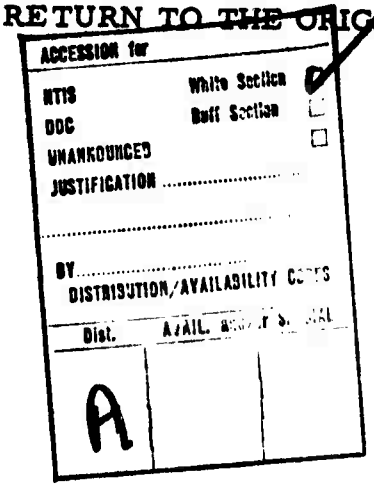

Letterman Army Institute of Research

Annual Progress Report, FY 1973

30 June 1973

ERRATA

Page 41, Item 23. (U) To discover and/or produce a topical repellent or repellent formulation against malaria-bearing mosquitoes and other vectors of militarily import diseases which will possess effectiveness as a repellent and will persist for more than twenty-four hours on human skin despite rub-off, sweating, and water wash-off. In-vitro testing methods are being used to determine physical properties of DEET and new repellent formulations to supplement the in vivo screening program. Interaction mechanisms between repellents and skin on additives are being studied to predict the evaporation and penetration rates of these formulations in vivo.

Page 57, Item 23. (U) Blisters of the feet cause considerable morbidity in soldiers on long marches. At one training camp 15 to 20 recruits are seen daily in the dispensaries for infected foot blisters and there are 3 to 6 recruits at all times hospitalized on the surgical service of the post hospital for complications of infected foot blisters. Utilizing experimentally produced blisters, the objective of this work unit is to study the reconstitution of the skin in terms of the barrier function following production of friction, thermal, and chemical blisters by studying the change in concentration of selective proteins with time after blistering.

Page 61, Item 23. (U) One of the most common of skin diseases in soldiers exposed to high heat and humidity is prickly heat. Its military importance is due not only to the discomfort it produces but it can usher in other disabling skin troubles, including folliculitis and furunculosis. It has been difficult to study prickly heat and its consequences in the soldier because the affected individuals are evacuated to cool environment or put at rest and therefore lose the principal manifestations of their skin disease. For this reason the disease is studied by experimentally produced prickly heat. This work unit proposes to determine the role of heat stress on protein and lipid synthesis in prickly heat resistant and prone individuals and to develop therapeutic counter measures against prickly heat.

Page 83, Item 23. (U) Warm immersion foot and its acute variant "paddy foot" causes severe disability in troops stationed in Southeast Asia, the Pacific islands, and has been a cause of man-days lost in soldiers stationed in artic areas. The purpose of this research is to define the clinical, histopathological, physical, biochemical, and immunological changes that result from prolonged exposure of skin to water and to guide the development of specific and effective prophylactic and therapeutic measures.

Page 93, Item 23. (U) Cutaneous fungal infections are common problems in military medicine. Under adverse environmental conditions like the hot and humid tropics, cutaneous fungal infections may become disabling to the individual, even rendering a division ineffectual. Presently no effective prophylactic or therapeutic agents are available that prevent cutaneous fungal infections from reaching epidemic proportions. The interaction between the infectious agent and the host is poorly understood, but it appears that the immunologic reactions these organisms initiate in man are significant in the host-parasite relationship. The purpose of this research is to study the immunologic relationships between these infectious agents in man.

Page 97, Item 23. (U) Fungal skin infections have been the leading cause of disability among ground combat troops in hot, wet areas of the world. Elucidation of the pathogenesis of these infections will contribute materially to developing effective methods of prevention. The objectives of this study are to: 1) Elucidate biochemical mechanisms of infection. 2) Perfect assay of enzymes capable of hydrolyzing substrates found in stratum corneum; characterize isolated enzymes by physical-chemical procedures. 3) Correlate virulence, enzyme production, and nutritional requirements of fungus with morphological and physiological variations in vitro and in vivo. 4) Determine antigenic activity of isolated enzymes. 5) Isolate and purify fungal antigens and fungal toxins. 6) Devise novel therapeutic and preventive measures by perfecting antigen isolation procedures for use in immunization studies.

Page 101, Item 23. (U) Fungal skin infections have been the leading cause of disability among ground combat troops in hot, wet areas of the world. The objectives of this study are to perfect a reproducible, experimental fungal infection of the skin of humans; to determine the minimum number of fungal spores required to produce persistent infections in humans with and without pre-existing immunity to dermatophytic fungi; to correlate the degree of immunity in human volunteers with the severity of the induced infections; to prepare a protective vaccine that will prevent or minimize the severity of

experimental and natural infections; and to evaluate prophylactic and therapeutic anti-fungal agents.

Page 137, Item 1. DA OC 6808

Page 181, Item 23. (U) 1) To increase in-house military laboratory capability in drug assay techniques. 2) To verify the significant adsorption and/or binding phenomena of digitoxin by steroid binding resins in vitro in order to more quickly and effectively reduce the levels in patients treated with digitoxin. 2) Produce metabolites of digitoxin in the beagle in order to study the role of these metabolites play in digitoxin intoxication and the manner in which they may be removed from the blood stream by resin adsorption.

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13. ABSTRACT

This report summarizes the investigative effort of FY 1973 at the Letterman Army Institute of Research. These studies are devoted to basic and applied research in skin diseases of military importance; the effects of hemorrhagic shock on the heart and brain; the development of techniques for the early management of complex maxillofacial wounds and injuries and the identification of diseases of the oral and maxillofacial tissues which are of special importance to the field soldier. Summaries of those clinical investigations at Letterman Army Medical Center involving collaborative studies or requiring major personnel, laboratory studies or animal resources support from LAIR are included.

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14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Skin						
Skin diseases						
Fungal infection						
Friction blisters						
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Blood-oxygen affinity curves						
Oxyhemoglobin dissociation						
Blood-gas transport						
Maxillofacial injuries						
Experimental animals						

AD_____

HEADQUARTERS
LETTERMAN ARMY INSTITUTE OF RESEARCH
Presidio of San Francisco, CA 94129

REPORT NO. 17

ANNUAL PROGRESS REPORT, FY 1973

1 July 1972 - 30 June 1973

RCS SGRD-288(R1)

FOREWORD

This report summarizes the investigative effort of FY 1973 at the Letterman Army Institute of Research. Significant new knowledge includes: low oxygen-hemaglobin disassociation values were found in intensive care unit patients following recent blood transfusion, heroin, Valium and Librium injections; a reliable method for producing cranial large vessel occlusion without associated infarction in sheep was devised; 3 new repellents persisting twice as long as presently available mosquito repellents were tested; acquired immunity to experimental skin fungal infections in man was demonstrated; improved methods for processing and sterilizing lyophilized allogeneic decalcified bone have been developed for mandibular bone grafts; the use of an oral lavage with a topical antiseptic significantly reduced the overall incidence of alveolar osteitis, and the incidence of alveolar osteitis in women taking oral contraceptives was nearly 3 times greater than in women not on the medication.

Summaries of those clinical investigations at Letterman Army Medical Center involving collaborative studies or requiring major personnel, laboratory studies or animal resources support from LAIR are included.

Phase I of the new, eventually 360,000 square feet LAIR laboratory, animal, and administrative buildings is 87 per cent complete; Phase II is 40 per cent complete, and the Phase III bid was opened in early June. It's fun to watch the foundations being dug, the walls being poured, and to see the buildings rise. Signing purchase requests for Class B and C items destined for the dream is a puzzling, informative, vicarious, and fun chore. Fortunately, a knowledgeable military project officer, an architect, and a supply officer are assigned to unravel much of the mystery. We eagerly await the arrival of fellow researchers from World War I buildings and tuberculosis wards to make our lab the best in the world.

William A. Akers

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IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Program Element 6.11.01.A

Project Number 3A061101A91C

Task Area Number 00

INDEPENDENT LABORATORY IN-HOUSE RESEARCH

The Independent Laboratory In-House Research Program (ILIR) consists of research the Commander considers worthwhile toward solution of military medical problems that are not funded in other programs. During the period of this report two investigations were terminated from the ILIR Program but the work will continue under other than RDTE funding. One investigation was completed and a final report of research is in preparation. Four investigations continue.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6809	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	H. Terminate	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

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a. PRIMARY	61101A	3A061101A91C	00	380
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Studies on Cerebral Pathophysiology In Early Hemorrhagic Shock (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 12	NA	DA	C. In-house

17. CONTRACT/GRANT

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18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 73	1	11.2
FISCAL YEAR CURRENT 74	0	0

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Hemorrhagic shock; (U) sheep; (U) cerebral blood flow; (U) blood-brain-barrier

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To correlate the clinical changes manifested in hemorrhage shock with electroencephalogram (EEG) changes, alterations in blood-brain barrier (BBB), alterations in cerebral blood flow and alterations in cardiac hemodynamics and retinal microvascular dynamics. To evaluate simple therapeutic modalities in hope of finding a simple way to protect the brain of humans who are suffering from hemorrhagic shock.

24. (U) Unanesthetized sheep with bilateral nonocclusive carotid catheters will be bled acutely into shock, observed, and retransfused. Basic EEG rhythm, BBB, cerebral and blood flow and retinal microvascular changes will be monitored serially and compared with changes in BP, P, EKG, cardiac output, blood gases and clinical parameters. Potential therapeutic agents will be administered intravenously and intracarotidarterially.

25. (U) 72 07 - 73 06 Thirty-one sheep were successfully studied and the changes observed in vital signs, EEG, brain scan, blood gases, histologic changes, occurring early in awake and fully viable sheep suggest these conclusions: 1) our incremental bleeding model better reproduces the clinical setting of acute blood loss. 2) There are early important changes in the brain in hemorrhagic shock.

This work unit is terminated and the research will continue under other than RDTE funding.

(Available in microfiche upon originator's approval)

Studies On Cerebral Pathophysiology In Early Hemorrhagic Shock

Michael M. Bronshvag, MAJ, MC

PROBLEM:

This study of cerebral changes in early hemorrhagic shock was begun in the search for something new, something simple that might be added to the standard emergency room and battalion aid station treatment of severe but remediable acute hemorrhagic shock.

APPROACH:

Critical of post studies using anesthetized dogs bled rapidly to a predetermined point and then artificially maintained there, we attempted to reproduce the clinical state by using unanesthetized sheep bled gradually over one hour period to severe shock. All sheep were conscious and fully viable. Sheep were prepared 1 - 3 days previously by insertion of arterial and venous catheters and the placement of burr-holes in the skull for obtaining biopsies. On the experiment day, sheep were given 50-200 cc of 1 per cent Evans blue (T1824) solution intravenously. Monitoring of blood pressure, pulse, EKG, respiration and EEG was done continually by standard techniques. Several sheep had several technitium 99^{m} pertechnitate-albumin brain scans and four had Doppler ultrasound estimation of superior sagittal sinus velocity (? flow).

Arterial, mixed venous, jugular venous and superior sinus blood was analyzed for blood gases and lactate concentration. Several brain biopsies were obtained on experimental and identically prepared control animals. Animals were sacrificed by carotid injection of four per cent paraformaldehyde and brains were removed and further plunged into paraformaldehyde. Fixation for study appeared to be excellent. Light microscopy, fluorescence microscopy (for Evans Blue-albumin complex) and electron microscopy were performed on biopsy and necropsy specimens.

RESULTS:

1. General: Thirty-one sheep were successfully studied.

2. Vital signs: As expected, during early HS, P increased, R increased 800 cc and BP was maintained. Further bleeding in the range of 40 cc/kg caused BP to drop to levels of 50/25 at which point the experiment was completed.

3. EEG during early bleeding, no significant changes appeared. At about 30 cc/kg, the range (5-7 cps) slow waves appeared. At 40 cc/kg, most (approximately two-thirds) sheep had slowing to delta (1-3 cps) range, but one-third only had minor changes. Some awake sheep (one-fourth to one-third) had bursts of 2-3 ps delta waves most prominent frontally, similar in configuration to that seen in uremic encephalopathy. No triphasic slow waves or spikes were noted.

4. Brain scan: No control sheep had an abnormal brain scan. During incremental bleeding to HS, the brain scan became progressively more positive in most (75%) sheep.

5. Blood Gases: Control valves were normal. Blood drawn at a point of severe HS showed slightly elevated arterial oxygen and pH, reflecting lung oligemia and overventilation. Venous O_2 and pH were markedly depressed and pCO_2 was elevated, reflecting tissue anoxia and overventilation. Venous O_2 and pH were markedly depressed and pCO_2 was elevated, reflecting tissue anoxia and acidosis. Arterial and venous lactates were elevated, reflecting hypoxia and anaerobiasis.

6. Histologic Changes: a) Biopsy and necropsy specimens showed no light microscopic changes. b) Fluorescence microscopy revealed no consistent differences between experimental and control animals in the distribution of Evans blue, although several individual HS specimens exhibited perivascular fluorescence compatible with gross leakage of blood-brain barrier. c) Electron microscopy, biopsy and necropsy specimens of the cerebral cortex at hemorrhagic shock showed the following differences from control specimens: (1) Swollen vacuolated mitochrondria in neurons.

(2) Edema of endfeet of pericapillary glial cells.
(3) No pathological change in endothelial cells or their nuclei.

DISCUSSION:

The described changes, occurring early in awake, fully viable sheep suggest these conclusions: 1) Our incremental bleeding model better reproduces the clinical setting of acute blood loss. 2) There are early important changes in the brain in hemorrhagic shock. In view of the neuronal mitochondrial and the alterations in superior saggital sinus pCO_2, pO_2, pH and lactate, the final mechanism is hypoxic and is similar to anoxic-ischemic lesions. However, since blood-brain barrier permeability is increased and histologic changes are demonstrated in glial cells known to be important in endothelial transport, a defect in endothelial transport is likely to be involved. It is emphasized that blood-brain barrier and glial perivascular changes of this nature are not seen in anoxic-ischemic lesions and that endothelial transport is not felt to be deficient in the hyperacute stages of anoxic-ischemic lesions. It would seem that a hyperactive metabolic lesion, not identical with simple anoxia-ischemia is present which interferes with appropriate transport of oxygen and nutrients.

This conclusion has two important spinoffs: 1) Since other organs do not have a blood tissue barrier, delicate early testing for gross derangements of transport function has been difficult. Scientists studying these organs and shock in general may be able to extrapolate our data to kidney, heart, liver and gut systems in their studies. 2) Since transport function can be enhanced by rather simple means (phenobarbital, corticosteroids, glucose and insulin), simple means of treatment HS are suggested.

FUTURE PLANS:

The incremental bleeding approach will be used in a small animal model. Using elevated cerebral tissue and superior sagittal sinus blood lactates as an end point, as well as EEG, several simple treatments will

be tried as outlined above in hopes of protecting the brain from the hyperacute metabolic lesion of hemorrhagic shock described in this report. A manuscript is being prepared in hopes of publication.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6791	73 07 01	DD-DR&E(AR)636

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72 07 01	K. Complete	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	382
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Characterization and Kinetic Studies of Discrete Classes of Granulocytes (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine; 002300-Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 03	NA	DA	C. In-house

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18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
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19. RESPONSIBLE DOD ORGANIZATION

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NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) leukocyte isolation; (U) leukopoiesis; (U) hematology

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Laboratory support of studies on cutaneous fungal infection, periodontal disease, would healing, burns, and allergic responses in soldiers.

24. (U) Leukocytes will be isolated from whole blood by agglutination of erythrocytes with dextran. The leukocytes will be separated on buffered, isotonic density gradients made with a high polymer of sucrose. Each cell type will be disrupted by shear or sonication, and the soluble and membrane-bound fractions will be injected into rabbits for the production of antibody.

25. (U) 72 07 - 73 06 Sufficient data has been obtained for definitive evaluation and two publications are in preparation.

*Available to contractors upon originator's approval.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6813	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	166
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Development of a Rapid Clinical Procedure for Assessing Blood-Oxygen Affinity Curves (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 09	CONT	DA	C. In-house

17. CONTRACT/GRANT
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c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

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FISCAL YEAR PRECEDING 73	.75	31.7
FISCAL YEAR CURRENT 74	.5	26.0

19. RESPONSIBLE DOD ORGANIZATION
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NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Blood-oxygen affinity curve; (U) oxyhemoglobin dissociation;(U)blood-gas transport

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) 1) To develop and evaluate a rapid, simple and reliable technique for determining the oxygen affinity of hemoblobin; 2) To investigate the role of oxygen affinity in modifying the physiologic response to combat-type trauma, environmental stress, and disease states; and 3) To discover agents with which benefically manipulate the oxygen affinity.

24. (U) Clinical trials of the new method for determining oxygen affinity.

25. (U) 72 07 - 73 06 Over 150 observations of seriously ill patients admitted to the Intensive Care Unit (ICU) at LAMC have been completed; newborn cord blood has been tested and patients with hemoglobin abnormalities studied. Further analysis of results with normal subjects has revealed a natural variability of oxygen affinity that may be useful in medical selection and physical training procedures for assignments involving hazardous duty or unusual fitness requirements. The curve shape (heme-heme interaction) has been found to be a major factor in this variability. Compared to normals, who display lowered oxygen affinity at low hemoglobin levels, ICU patients had no consistent relationship between these variables. Oxygen partial pressure at half-saturation of hemoglobin (P_{50}) fell between 26 and 29mm Hg in normals; ICU patients ranged from 20 to 37mm Hg, high values being rare. Low P_{50} in the latter group was commonly associated with either a recent blood transfusion or use of the tranquilizers Valium and Librium. The apparent effect of Valium and Librium on oxygen affinity has not been reported previously and may represent an important clinical feature in the prevalent use of these drugs. Two cases of reported heroin overdose were also associated with a significant increase in oxygen affinity.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Development of a Rapid Clinical Procedure For Assessing Blood-Oxygen Affinity Curves

J. Ryan Neville, Ph.D., Research Physiologist

PROBLEM:

From a technical standpoint, the objectives and background of this study have been described in the FY 1972 Annual Progress Report. As documented in the "Results" section of this, as well as the previous report, these objectives have been effectively accomplished and the procedure for measuring oxygen affinity that has evolved from this effort appears to meet all the criteria originally proposed for such a test; that is, rapidity and simplicity, require negligible quantities of blood, utilize existing clinical instrumentation, and have sufficient accuracy and reproducibility to reflect meaningful physiologic variation in oxygen affinity. If the assessment of oxygen affinity was a widely used diagnostic aid in medical practice, achievement of the above technical objectives would represent a logical final goal for the present experimental efforts. Only recently, however, has the potential importance of oxygen affinity in both health and disease attracted the interest of clinicians; and the evidence bearing on the problem, while theoretically suggestive, is neither extensive nor conclusive. Consequently, the advantages of the new technique should be more convincing if, in addition to the technical improvements, its utility can be demonstrated by actual application to clinical problems which might reasonably benefit from more complete oxygen transport information. Therefore, the current and future objective of the present work will be directed toward the actual application of the developed technique to a variety of clinical problems with the aim of demonstrating the practical, as opposed to the mere theoretical importance of changes in oxygen affinity in humans.

APPROACH:

Using the technique described in previous reports under well controlled conditions (temperature, pH, base excess, carbon dioxide, anticoagulant, etc.), attempts

have been and will continue to be made to define the actual variation in oxygen affinity that can be encountered under normal healthy conditions as well as in certain disease states. While in most cases, the shape and position of the curve under standard conditions constitutes the major variable of interest, in some instances it is desirable to ascertain how acid-base changes (both respiratory and metabolic) affect oxygen affinity. The effect of pH on oxygen affinity (the so-called Bohr effect) has been found to differ depending on whether pH changes are respiratory or metabolic in origin. While such differences are apparently rather small in the normal pH range encountered in health, they can be sizable in acidosis and perhaps in alkalosis as well. Furthermore, acidoisis, which decreases oxygen affinity and thus favors oxygen unloading in tissues, may under certain circumstances, tend to deplete erythrocytes of 2, 3-DPG. Consequently, the sudden correction of an existing acidosis by, for instance, infusion of bicarbonate might severely compromise the oxygen transport system through the effect that alkali and lowered 2, 3-DPG have on increasing oxygen affinity.

Attempts to determine the extent of the natural variation in oxygen affinity that occur in health and disease constitute a survey, the aim of which is to determine under what conditions such changes occur, how large an effect is apparent, and whether or not the variation encountered might reasonably be expected to have any physiologic significance to the organism. In conjunction with such a survey, in vitro experiments have been performed for the purpose of ascertaining to what extent certain drugs as well as other factors can alter oxygen affinity. Ultimately, of course, any knowledge gained from investigation of the significance of oxygen affinity changes would perhaps have its greatest expression in the ability to manipulate the dissociation curve in order to bring about desired effects at will. Efforts to identify agents capable of inducing such transformation will continue.

RESULTS:

Analysis of additional oxygen affinity data of ten hematologically normal adult males has indicated that oxygen affinity is specifically influenced by carbon

dioxide independently of pH shifts induced by changes in partial pressure of this gas. The carbon dioxide Bohr factor was found to be -.53 (±.01), in good agreement with the findings of Wranne, et al. (1). Also in agreement with the latter investigator was the finding that the carbon dioxide Bohr is not affected by change in the base excess. On the other hand, when pH shifts were induced by change in the ratio of the Tris buffer constituents normally used with the oxygen affinity technique, the Bohr factor was smaller and varied considerably depending on the base excess value. Extreme negative values of the base excess (pH levels of 7.0 to 7.25) yielded very small Bohr factors in the range of -.20, this value increasing toward the carbon dioxide Bohr factor as pH rose with the use of less negative base excess. This finding of a non-linear effect of base excess on oxygen affinity apparently explains previously unresolved discrepancies in the experimental data bearing on this problem. At least, it has been possible, by comparison of the nature of the Bohr effects found in the present experiments with the presumably conflicting data in the literature, to harmonize most of this information.

The data on normal subjects have indicated that the group variation of P-50% (partial pressure of oxygen at half saturation), when corrected to 7.4 and 0 base excess, is relatively small, consistent with previous results. In one series of experiments in which 3 trials were performed on each of 10 individuals, a mean of 27.7 mm Hg (±.7) was found with an actual range of 26.4 to 29.5 mm Hg. This series also yielded interesting and previously unreported information regarding the heme-heme interaction or n-value for these normal individuals. While the group mean for n was 2.56 (±.13), similar to the value generally reported for normals, an analysis of variance indicated a ratio of group variance to individual variance of greater than 10 ($n:1 = 9$; $n:2 = 20$) with a P value of less than .001. Surprising also was the suggestive positive correlation (.4) found between P-50% (corrected) and n. The range of n values for the group in this series was 2.30 to 2.81. These findings are discussed in the next section. Perhaps such results were observed because of the unique capability of the yeast technique for tracing the entire dissociation curve in a matter of a few minutes. Since the n value depends on the ratio of the difference in two sets of values (log P-50% and log % Sat/100-% Sat) derived from this curve,

experimental errors tend to be cancelled, unlike the situation with P-50% which is sensitive to random experimental error. This being the case, the values found for n may represent to a large extent the true normal variation of this factor whereas with P-50% the range found is likely to be composed of random experimental error as well as normal variation. In fact, if some of the experimental errors known to be involved in the P-50% measurement are considered, these appear to account for a large part of the observed variation. If instead of the observed P-50%, an estimate of P-50% is made from the regression equation found in the correlation analysis of P-50% and n, a smaller range of P-50% values (27.2 - 28.3) is predicted for the present group of normal subjects. Assuming the arguments regarding the accuracy of n as measured with the present technique are correct, this latter range of P-50% values may be representative of the true normal variation in this measure of oxygen affinity. While seemingly small, evidence has been published (2) indicating that a change over this range would require a ten percent alteration in 2, 3-DPG.

Although analysis of observations made on patients admitted to the Intensive Care Unit of Letterman Army Medical Center has not been completed, it is possible to report a number of preliminary findings that have been regularly associated with this group. A major difficulty in analyzing the large amount of data available is the great diversity of clinical material included in this series. Originally it was speculated that this diversity might enable one to identify special disease entities in which the status of the oxygen transport function played a particularly crucial role in the condition of the patient. The analysis to date has not supported this expectation and it appears more likely that any role played is highly generalized without specific diagnostic value (an exception to this is already apparent in the use of oxygen affinity information to characterize certain hemoglobinopathies).

Compared to the small range noted in normals, corrected P-50% values found in ICU patients have been prominently scattered over a wide range from a low of about 20 mm Hg to, in one case, as high as 37 mm Hg. Values below the lower limit of the normal range previously noted (26.4 to 29.5) far exceeded those found above this range.

Although the mean P-50% of the series (approximately 24.5 mm Hg) is significantly different from the normal, the frequency pattern of these values is not normally distributed so that this comparison is not valid even though the pattern of values found in this group is distinctly abnormal.

A plot of hematocrit values versus P-50% (corrected) failed to display the inverse relation between these variables that was previously reported for healthy adult males. Although the ICU patients observed included a large number of females, low or high values could not be segregated on the basis of sex. A striking finding was the regular association of low P-50% values in this series with either recent blood transfusions or with the reported use of the tranquilizers Valium and Librium. The highest value noted in the series, 37 mm Hg, was obtained in a patient receiving massive doses of steroids (methyl prednisolone) for treatment of an acute and massive myocardial infarction. An initial attempt to confirm the apparent effect of tranquilizers by incubating normal blood with Valium in vitro resulted in an increased oxygen affinity (P-50% decreased an average of 3 mm Hg). This result is based on only 3 observations on a single blood sample and further experimental work is required to confirm the nature and extent of the change.

Some preliminary observations on cord blood of newborn infants has confirmed that the position of the curve (P-50% = 20 mm Hg) is shifted to the left. The difference in the positions assumed by the fetal and maternal hemoglobin has been recognized as an important mechanism in the transfer of oxygen from the placenta to the fetal circulation and any decrease of the oxygen partial pressure gradient that exists between these curves would be expected to limit the efficiency of oxygen transport. In view of this, the observation reported above on the effect of tranquilizers in lowering P-50% may be of particular importance in obstetrical situations where use of such drugs is fairly prevalent. Of equal significance, the experimentally observed Bohr shift in cord blood was exaggerated (Bohr factor = -.75) compared to the usual adult value. While this enhanced Bohr effect might aid the unloading of oxygen in fetal tissues, at the same time it would cause the maternal to fetal oxygen transport process to be especially vulnerable to acidosis, since this condition would tend to lower the normal oxygen gradient existing between the placental sinus and the fetal villi.

DISCUSSION:

In most respects, the data obtained in hematologically normal subjects has been similar to that published elsewhere. The specific response to carbon dioxide, the P-50% values, as well as the n values (heme-heme interaction) are all in good agreement with recently published accounts. The additional findings relating to the precise nature of the base excess Bohr effect, the significant differences found in individual n values, and the apparent correlation between individual P-50% and corresponding n values are unexpected new findings which require confirmation. Edwards and Rigas (3) have examined the oxygen affinity characteristics of human red blood cells separated on the basis of *in vivo* age by ultra-centrifugation. They found that younger cells had both a higher P-50% and a higher n value than did the older cells. If these latter observations could be shown to be the basis of the apparent relationship between n and P-50% found in the present series, the clinical value of oxygen affinity measurements might gain additional stature since present techniques for examining the *in vivo* age of erythrocytes have several disadvantages.

Presently one can only speculate on the possible physiologic significance of the variation found in normals. The individuals used in this study constitute a small and fairly homogenous sample of young, healthy, adult males. Even so, the differences observed, particularly in the heme-heme interaction, might hypothetically be suspected of altering certain functional capabilities to some extent. Night vision, for instance, is known to be extremely sensitive to moderate degrees of hypoxia and performance even at low altitudes can be compromised on this basis. Other things being equal, it would seem reasonable that individuals with the more rightward position of the curve could retain their night vision effectiveness to a significantly higher altitude than could those with the more leftward displacement. Although its true physiologic significance is no more firmly established than is the case with normals, the sheer range of values found in the ICU patients seemingly offers more plausible grounds for speculation. One of the lowest values in this series (P-50% = 20) was a young negro woman who had experienced a difficult childbirth. She had an extremely low hematocrit when admitted to the ICU and had received Valium as a preanesthetic sedative. Although multiple transfusions presumably alleviated the immediate crisis, they

probably also contributed to the leftward shift in the oxygen affinity through the Valtis-Kennedy effect. The apparent effect of the transfusions on oxygen transport was estimated to be about a 20 per cent improvement. If the oxygen affinity had been normal, it was estimated that the patient would have experienced more than a 100 per cent improvement in oxygen transport even without the transfusion. In this series low hematocrits were not, as previously indicated, invariably associated with high oxygen affinity, and many of the patients appeared to have effectively compensated for low circulating red mass with a rightward swing in oxygen affinity. In such instances, it is questionable that transfusion would be justifiable solely on the grounds of improving oxygen delivery to tissues. In fact, unless fresh blood was used, a transfusion in such cases would usually result in a net decrease in tissue oxygenation by increasing oxygen affinity. Since several drugs (Valium, propranolol, steroids) are now known with which it appears possible to manipulate the oxygen affinity, it is likely that a much more practical experimental assessment of the physiologic significance of oxygen affinity changes will be available soon.

FUTURE PLANS:

We plan to continue with the same general approach that has been used in the past. Clinical material from the hospital will be examined to further define the limits of variability that are present and to gain insight into the possible causes and significance of these changes. Experiments, possibly on normal volunteers, will be carried out to confirm the retrospective findings with regard to tranquilizers. Further in vitro screening of drugs having potential activity with relation to oxygen affinity will also be performed. If suitable experimental facilities can be found, an attempt will be made to define the effect of oxygen affinity on decrements in night vision at altitude or with hypoxia.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6926	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	611101A	3A061101A91C	00	387
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Pathogenesis of Coliform Induced Colitis in Mice (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine; 010100 - Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 03		DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	73	.1	3.3
	CURRENT 74	.1	5.0

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

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ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) pathology

(U) Experimental animal; (U) model; (U) mice; (U) colitis in mice; (U) bacterial colitis;

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To develop an animal model using mice for producing chronic ileitis and colitis since diarrheal diseases, especially in tropical areas, rank second in cause of man days lost in soldiers.

24. (U) Barrier raised Swiss Webster mice were exposed to a washed saline suspension of coliform agent [Citrobacter freundii (ANL)]. To study the clinical, gross and microscopic features, animals were serially killed over a 4 month period and examined. Light microscopic changes were compared to those observed in idiopathic ileitis and colitis of man.

25. (U) 72 07 - 73 06 Lesions produced were striking and remained active 3 to 70 days post inoculation. Many features found in exposed mice were like those seen in chronic idiopathic ulcerative colitis in man including marked epithelial hyperplasia, mucosal degeneration and ulceration, downgrowth and isolation of colonic glands in the submucosa.

* Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

Pathogenesis of Coliform Induced Colitis in Mice:

Robert M. Kovatch, LTC, VC

PROBLEM:

This study was designed to document the sequential development of the lesions of the digestive tract of mice exposed to a coliform bacterium classified initially as a lysine decarboxylase negative Enterobacter hafnia. This agent has now been reclassified as a Citrobacter freundii (ANL) by the National Animal Disease Laboratory, Ames, Iowa. The agent was obtained from the National Institutes of Health and was originally isolated from a mouse during a spontaneous outbreak of colitis at Fort Detrick, Frederick, Md.

The objective of the study was to document the morphologic changes in exposed mice and to compare the lesions to those of chronic idiopathic ileitis and colitis of man. It was hoped this small laboratory animal system might be developed to study the complex mechanisms active in the inducement of chronic idiopathic ileitis and colitis of man.

APPROACH:

The initial study will be divided into 2 phases (1) Cesarean derived barrier maintained Swiss Webster mice will be exposed to 3 levels of saline washed C. freundii in a saline suspension by gavage. From these 4 groups, animals will be randomly selected and killed at weekly intervals for 5 consecutive weeks. Clinical features and gross and microscopic alterations will be evaluated to determine the most favorable dose of coliform agent to reproduce the disease (2) utilizing information derived from phase 1 of the study additional barrier maintained mice from the same source will be exposed by gavage to a dose of C. freundii calculated to induce colonic lesions with a minimum of mortality. Five coliform treated animals will be killed on 1, 3, 6, 9, 12 days post inoculation (PI). Additional animals will be killed at weekly intervals 2 thru 10 weeks and on the 12th, 16th and 20th weeks PI. Sham treated animals will be given a like quantity of normal saline by gavage and killed on the same dates as the coliform treated, to serve as

controls. Non-treated animals will be killed to collect material for histology and for bacterial culture to determine normal intestinal flora. A portion of the mid-colon will be aseptically removed, the mucosa scraped and material streaked on MacConkey's and tryptocase soy-blood agar plates for C. freundii recovery. Material will be collected from the distal colon and fixed for electron microscopy. Specimens will be taken from 3 levels of the colon, cecum, 3 levels of intestine and other peritoneal and thoracic viscera for fixation in neutral buffered formalin for light microscopy.

RESULTS:

Phase I of the study included a clinical, gross and light microscopic study of groups of mice exposed to washed saline suspensions of organisms of 3 different optical densities (0.10, 0.61 and 0.85). Analysis of data from this preliminary study indicated a dose of 0.1 cc of bacterial suspension that measured 0.61 OD was optimum. Excellent histologic lesions with a minimum of mortality was found.

Phase II

Clinical Features: Clinical signs that consisted of a moist stool, perianal mucus deposition and hyperemia and swelling of the perianal ring were initially detected 14 days PI and last detected 28 days PI. When the animals remaining at 17 days PI were individually examined most of the treated animals had clinical signs. The first animal with a rectal prolapse was noted 21 days PI. Several other animals prolapsed during the subsequent 14 days. During this period straining without defecation was noted in some.

Gross Lesions: Lesions were limited to the colon, rectum and mesenteric lymph nodes. The first were seen in the colon of 2 of 5 examined 9 days PI and consisted of mild enlargement of the distal portion. By 21 days all the animals had marked megacolon (3-5x normal) of the distal segment and moderate enlargement of the mid and proximal colon of some. The unopened involved segment was turgid and opaque. On cross section, the wall was thickened, mucosa folded and the lumen was of reduced diameter. The mucosal surface was irregular and folded. Hyperemic linear ridges were seen in some.

The megacolon became less obvious during the course of the experiment but was still observed on the last kill date. Enlarged mesenteric lymph nodes were first noted 4 weeks PI and remained enlarged in animals for several weeks. The protruding segment of rectal mucosa in animals that had prolapsed remained viable during the entire course of the study even in those killed near the termination of the experiment.

Microbiology: Recoveries of C. freundii from treated animals were obtained from scrapings of colonic mucosa streaked on MacConkey and/or tryptocase soy-blood agar plates from 9 thru 49 days PI. Following 3 weekly negative cultures isolation attempts were discontinued. All cultures taken from colonic mucosa of non-treated and saline shams during the course of the experiment were negative for C. freundii.

The sequential development of lesions in the colon can best be described as acute 3-12 days PI, subacute 14-28 days PI, active chronic 28-70 days and residual lesions between 77 and 120 days PI. There was some overlapping of lesions and individual animals were out of sequence i.e. acute case in 14-28 day group; therefore, the groupings are somewhat arbitrary. In all cases the lesions were most severe in the distal colon with less severe lesions in the mid and proximal colon. The earliest acute lesion seen in 2 of five animals was detected 3 days PI. The lesion consisted of stasis of neutrophils in dilated vessels of the lamina propria of the distal colon. In one animal killed 6 days PI large numbers of coccoid bacteria were noted at the luminal surface of the distal colon. Adjacent epithelial cells had degenerated. In all animals stasis of neutrophils in dilated vessels of the lamina propria, numerous inflammatory cells caught in transmigration of the mucosa, and degeneration of individual or small groups of epithelial cells were noted. By 12 days PI features included marked epithelial degeneration, focal epithelial erosions, reduction in number of goblet cells, occasional crypt abscesses, infiltration of neutrophils and edema of the lamina propria and submucosa, and marked focal proliferation of small coccoid bacteria at the luminal surface.

The salient histologic feature in the subacute group was marked epithelial hyperplasia. The lesion

was characterized by numerous mitoses of epithelial cells near the base and midway up the colonic glands, depletion or absence of goblet cells, and pseudostratification with frequent excessive extrusion of epithelial cells at the luminal surface. In severe cases the height of the mucosa was excessive compromising the lumen; glands were compact, and the glands at the luminal surface were flattened (mushroom shaped) and distorted. Other features included crypt abscesses, cryptitis with interruptions in the muscularis mucosa and in a few mucosal ulceration. Lymphoid hyperplasia was noted in the submucosa and in the mesenteric lymph nodes.

Features of active chronic inflammation included marked connective tissue proliferation and infiltrates of neutrophils and lymphocytes, and mucosal ulceration with down growth and isolation of colonic glands in the submucosa. The goblet cell population was less depleted or had returned to normal in some. Lymphoid tissues of the mucosa, submucosa and mesenteric nodes remained hyperplastic.

Residual lesions included small mucosal scars, occasional mucosal cysts and hyperplastic lymphoid tissues. Some of the animals had rather diffuse fibrosis of the lamina propria. In other animals, no lesions were found in the sections examined.

DISCUSSION:

The lesions produced with the C. freundii (ANL) produces severe hyperplastic and degenerative lesions of the mucosa of the colon. Lesions were most severe in the distal third of the colon with less severe lesions in the mid and proximal colon. Although onset of clinical signs and inducement of lesions was more rapid at the higher dose levels little alteration in mortality was found.

The mechanism for the marked epithelial stimulation and degeneration has not been determined at this time. An interesting hypothesis to consider is the possible production of nitrosamine by this agent. The C. freundii used in the present investigation has many common biochemical characteristics to members of the proteus group including hydrolysis of urea and decarboxylation of ornithine. Nitrosamine production by Proteus mira-

bilis has been shown both in vivo in man and in vitro systems. These compounds are known to stimulate marked cellular hyperplasia and at appropriate levels shown to be potent chemical carcinogens.

Although the usefulness of this system as a laboratory model for the study of chronic colitis has not been firmly established at this time, initial results were encouraging. The mechanism for epithelial stimulation and degeneration and host range need to be defined.

FUTURE PLANS

Examination of gluteraldehyde fixed specimens collected from mice during investigations this year will be processed and examined when the electron microscopy facilities are completed in Phase I LAIR.

Nitrosamine determinations in an in vivo system will be done using heat treated cultures of C. freundii. A gas chromatograph will be used for the chemical determinations.

The host range of the organism will be determined using weanling hamsters, rabbits, rats, and guinea pigs. Clinical, gross and histologic examinations will be done.

Seitz filtered saline suspension of C. freundii will be passed into mice to eliminate a possible viral etiology.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6927	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	H. Terminate	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	611601A	3A061601A91C	00	388
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Detection of (Superior Sagittal Sinus) Thrombosis (In Dogs) Using Radionuclide Labelled Fibrinogen (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 03	NA	DA	C. In-house

17. CONTRACT OR GRANT:
a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

16. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
73	1	3.9
CURRENT 74	0	0

18. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE: DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) cranial venous sinus occlusion; (U) I-131-tagged fibrinogen; (U) large vessel occlusion

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To develop an animal model to study the role of blood-brain-barrier in irreversible shock due to hemorrhagic shock as occurs in combat. Factors of sludged blood and thromboses are introduced. To detect occlusions (thrombi) in large vessels before frank brain infarction has supervened - specifically in superior sagittal sinus caused by closed or penetrating head injury, infection or dehydration.

24. (U) To induce thrombosis in sheep superior sagittal sinus and detect them using 123-iodine labelled fibronogen and the Pho Gamma III camera.

25. (U) 72 07 - 73 06 1) Fibrinogen has successfully been labelled with 131-iodine by both chloramine-T and iodine monochloride techniques. 2) Superior sagittal sinus thrombosis, unaccompanied by tissue hemorrhagic infarction or coagulative necrosis has reliably and reproducibly been induced by electrocoagulation. 3) Adequate virtual imaging has been achieved with the Pho Gamma III camera.

Work unit is terminated and research investigation will continue under other than RDTE funding.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

Detection of (Superior Sagittal Sinus) Thrombosis (In Dogs) Using Radionuclide Labelled Fibrinogen

Michael M. Bronshvag, MAJ, MC

PROBLEM:

Occlusion of large vessels accompanied by frank brain infarction is not difficult to diagnose. High-grade occulsion without current infarction is a much more crucial state because diagnosis is much more difficult, infarction may occur at any moment (often with disastrous or fatal issue), and yet the opportunity for the most gratifying result (prevention of infarction) exists. Superior sagittal sinus occlusion following dehydration, head trauma, cranial penetration or infection as well as several conditions not related to battlefield or training field medicine (pregnancy, birth control pills, etc.) is a classical prototype of this situation. Occlusion is difficult to diagnose without angiography (which carries its own risk) and the untreated condition may terminate with fatal hemorrhagic infarction. Other vessels with similar propensities include coronary and carotid vessels. We felt that radioiodinated fibrinogen might enter into the unstable thrombi that exist in these high flow vessels, and be amenable to imaging. ^{125}I and ^{131}I labelled fibrinogen have had some use in the point-source localization of leg clots (1), but are unsuitable for Pho Gamma camera imaging in humans, because of their long half-lives and high percentage of alpha and beta emissions. The head and heart, likewise are not readily amenable to point source localization. With the development of ^{123}I (2), a cyclotron-produced pure gamma emitter with a 13 hour half life, Pho Gamma imaging of intravascular thrombi seems feasible.

APPROACH:

Fibrinogen was extracted from ovine plasma by precipitation in 25% (NH_4) SO_4 and redissolution in normal saline (3). Clottability as measured by colorimetric protein determination was 90 - 95% (3). ^{131}I was complexed to the purified fibrinogen by chloramine - T method (4) or Iodine monochloride method (3).

Tagging of fibrinogen with Tc^{99m} pertechnitate by zirconium electrolysis was attempted (5). Tc^{99m} is also a pure gamma emitter of short half life - 6 hours).

Sheep were prepared as follows: The superior sagittal sinus was exposed and a thin stainless steel wire was passed into it and advanced 1 - 2 cm. The wound was closed with a small amount of the stainless wire exposed. One to three days later, the wire was attached to the Bovie coagulator and the sheep was grounded. Parasagittal EEG electrodes were placed. A Bovie coagulation current of 2 (scale of 10) was passed through the wire for 15 second periods with 15 second rest periods. When the parasagittal EEG leads changed (rather suddenly) from normal rhythms to high voltage slow rhythms, superior sagittal sinus occlusion was considered complete (6).

On 25 April 1973, we received permission from SGO to commence using cyclotron-produced ^{123}I. (We were loath to proceed with ^{131}I because the doses required for adequate Pho Gamma imaging would result in significant radiation exposures while making and administering the fibrinogen.)

RESULTS:

1. Tagging efforts with ^{131}I resulted in 50% tagging of the ^{131}I (very acceptable) to a 90% still clottable fibrinogen motility (very acceptable).

2. Tagging efforts with Tc^{99m} were unsuccessful.

3. Superior sagittal sinus thrombi without ischemic (infarctive) or coagulative (electrical-heat) necrosis were reliably produced by the above method, as judged by necropsy examination.

DISCUSSION:

A reliable method for producing large vessel occlusion without associated infarction (and secondary conversion of large amounts of fibrinogen to fibrin) has been devised. Chemical preparation of a satisfactory gamma-emitting radionuclide of fibrinogen can be accomplished.

FUTURE PLANS:

With recent SGO approval, we will purchase ^{123}I, prepare

^{123}I fibrinogen and complete the study. By administering the fibrinogen either before or after production of a superior sagittal sinus clot, and by drawing and counting of appropriately timed samples of blood (RBC, clot and serum) we hope to study fibrinogen kinetics as well (7,8).

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6928	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	389
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) The Relationship of Polymorphonuclear Neutrophil (PMN) Chemotactic Activity to Inflammatory Periodontal Diseases in Military Personnel (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 03	74 07	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	1	3.1
	CURRENT 74	1	7

19. RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Institute of Research
ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL
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TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION
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TELEPHONE: 415-561-4042
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Hutchinson, R.A., LTC DC
NAME: Lilly, G.E., COL DC

21. GENERAL USE

DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U)periodontal disease; (U) periodontitis;(U)inflammation; (U) chemotaxis;(U) polymorphonuclear neutrophil; (U)white blood cell;(U)bacteria

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) 1) Determine the variability of the WBC chemotactic response in military personnel. 2) Determine relationship between WBC chemotactic response and inflammatory periodontal disease. 3) Determine the WBC chemotactic activity of various saliva components with fresh serum.

24. (U) White blood cell (WBC) migration (chemotaxis) will be measured in the laboratory using a Boyden chamber. The WBC chemotactic activity of cells from patients with severe inflammatory periodontal disease will be compared with the chemotactic activity of cells from healthy controls. The chemotactic stimuli will be various mixtures of whole saliva and pure parotid secretion plus fresh homologous and heterologous serum.

25. (U) 72 07 - 73 06 Human subjects with severe periodontal disease and healthy control subjects have been evaluated for WBC chemotactic activity. The WBC chemotactic activity of cells from subjects with severe inflammatory periodontal disease was not different from WBC chemotactic activity from healthy controls. Likewise there were no apparent differences in complement dependent WBC chemotactic factors produced in sera from severe periodontal disease subjects or controls. However it was observed that both whole saliva and pure parotid secretion produced marked WBC chemotactic activity when reacted with fresh serum. The interaction of saliva components and serum is being further investigated as a possible component in oral inflammatory disease and wound healing of oral tissues.

* Available to contractors upon originator's approval.

The Relationship of Polymorphonuclear Neutrophil (PMN) Chemotactic Activity to Inflammatory Periodontal Diseases in Military Personnel

Major Thomas R. Tempel, DC
SP4 Henry L. Lazarus
Barbara A. Cheney
Colonel Gilbert E. Lilly, DC

PROBLEM:

The primary objective of this study is to evaluate polymorphonuclear neutrophil (PMN) chemotactic response as a possible laboratory means of detecting the degree of inflammatory periodontal disease in a soldier. Our hypothesis is that variations occur in the chemotactic response of polymorphonuclear leukocytes in individuals, who are otherwise "normal"; and such differences account for variations in individual susceptibility, resistance, and response to infectious inflammatory diseases. The chemotactic response can be measured in the laboratory and variations in PMN chemotaxis can be correlated with the severity of inflammatory periodontal disease.

Present evidence favors the concept that oral bacteria are the most important etiologic agent in periodontal disease. The bacteria colonize as dental plaque adjacent to the gum tissue. PMNs are found in the normal gingival sulcus as well as the periodontal disease lesion adjacent to the dental plaque and are thought to play an important role in the patient's resistance to periodontal disease. Patients with reduced numbers of PMNs or PMN chemotaxis defects are prone to severe destructive periodontal disease.

Recent studies have established that PMN chemotaxis defects occur in some individuals. Such individuals have altered inflammatory responses and are predisposed to infections. This abnormality has been recognized in specific diseases involving children and adults. In view of recent findings concerning chemotaxis, this defect may conceivably be a factor in the etiology and course of other inflammatory diseases. Current laboratory procedures offer a method for evaluating PMN chemotaxis which could prove of value in determining etiology, course, and patient susceptibility to inflammatory diseases, and provide methods for monitoring and diagnosing such diseases.

In 1962 Boyden developed an in-vitro method of studying cell migration which is sensitive, reproducible, and yields quantitative data relative to PMN chemotactic response to a variety of chemotactic factors.

Studies during the past 10 years have clearly established the following:

1. PMNs respond to a chemotactic substance by unidirectional migration toward the greatest concentration of the attractant substance (chemotaxis).

2. Most bacteria elaborate a product that is directly chemotactic for PMNs. It is not known if fungi or mycoplasma elaborate a similar chemotactic product.

3. Antigen-antibody complexes (IgG and IgM) generate a chemotactic factor by activating complement. This factor has a molecular weight of 15,000 and is derived from C5 with the active fragment C5a.

4. PMNs from patients with Chediak-Higashi disease and diabetes have been shown to be defective in their ability to respond to chemotactic stimuli.

5. Recent evidence suggests that patients with inflammatory peridontal disease or stomatitis may show variations in PMN chemotactic response when compared to controls.

APPROACH:

Our hypothesis was tested by correlating the periodontal disease index with the PMN chemotaxis index. The experimental methods and controls are outlined below.

Nine patients with severe periodontal disease served as subjects in this study. Nine additional individuals without periodontal disease served as healthy controls.

All subjects were involved in the following experimental procedure:

1. Peridontal Disease Index:

a. Personal data was recorded: age, rank, social security number, sex, and a brief medical and dental history.

b. Clinical dental examinations were given and the degree of periodontal disease measured using the Ramjford periodontal disease index. A sample of whole saliva was collected and frozen.

2. PMN Chemotaxis Index:

a. Twenty(20) ml of blood was drawn from the antecubital vein into a heparinized tube. Ten (10) additional ml of blood was used to prepare fresh serum.

b. The heparinized blood was sedimented in a 2 percent Dextran solution. The plasma was separated and PMNs were diluted in Gey's solution. Cells were washed twice and concentration adjusted to 2.2×10^6 cells per ml. The final suspension of PMNs was placed in the upper part of the Boyden chamber after the chemotactic substance was placed in the lower part.

c. The serum was removed from the clotted blood and frozen if not used immediately.

d. Chemotactic stimuli:

(1) Pooled autoclaved whole saliva was the source of Bacterial Chemotactic Factor.

(2) Endotoxin activated fresh serum was the source of complement dependent chemotactic activity.

(3) 0.2 ml of fresh whole saliva was used to activate fresh serum chemotactic activity.

e. Millipore filters were numbered and placed in the Boyden chamber.

f. The chemotactic stimuli or control were placed in Gey's solution in the lower part of the Boyden chamber; the PMN suspension in the upper part.

g. The following test substances were used to evaluate WBC chemotactic activity in cells from both diseased and control subjects.

Chemotactic Substance

1. Bacterial CTX (whole sterilized saliva)
2. Endotoxin activated serum
3. Heat inactivated serum plus endotoxin (control)
4. Whole saliva activated serum
5. Heat inactivated serum plus whole saliva (control)
6. Gey's medium control

h. The chambers were incubated for three hours at 37°C, 5 percent CO_2, in high humidity.

i. Filters were washed, stained with hematoxylin, cleared and mounted on microscopic slides with a cover glass.

j. Cells were counted on the bottom of the filter discs using 450X magnification and a grid. Ten random fields were averaged. The average number of PMNs per high power field was the chemotactic activity. The chemotactic activity minus the background cell migration was the chemotaxis index. Reading of slides was done in a single blind manner on coded slides. When the chemotaxis values were established, the values were decoded.

RESULTS:

Human subjects with severe inflammatory periodontal disease and healthy control subjects were evaluated for PMN chemotactic activity. The PMN chemotactic activity of cells from subjects with severe inflammatory periodontal disease was not different from PMN chemotactic activity from healthy controls. Likewise, there were no apparent differences in complement dependent PMN chemotactic factors produced in sera from either the disease group or controls.

DISCUSSION:

Under the conditions of the present study, it is readily apparent that PMN chemotactic function is not a valid parameter for screening patients with severe periodontal disease. However, it was observed that whole saliva produced marked PMN chemotactic activity when reacted with fresh serum. Further experiments with pure parotid secretions revealed that the pure secretion also activates fresh serum producing chemotactic activity, but to a smaller degree less than whole saliva. This finding represents the first indication of a possible interaction between homologous saliva components and tissue fluids which may be of significance in oral inflammatory disease and wound healing of infected oral tissues.

FUTURE PLANS:

Studies will be conducted to identify the various salivary components that react with fresh serum to produce PMN chemotactic activity. The serum component that is activated to produce the PMN chemotactic activity will also be identified.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6929	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10 NO 'CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	39
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Some Effects of Hypertonic Medium on Cultured Mammalian Cells (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 04	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 73	.5	6.8
FISCAL YEAR CURRENT 74	.5	21.9

19. RESPONSIBLE DOD ORGANIZATION

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TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) cell growth; (U) cell division; (U) stimulation of cell growth

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Stimulate cell division for application to faster wound healing. A cell culture system will be used to investigate the effect of nontoxic additives to the culture medium. Quantitate changes effected by hypertonic treatment in mammalian cells relating to: sulhydryl-disulfide ratios; mitotic index; macromolecular synthesis; ultrastructure; and induction of division as an approach to enhancement of wound healing.

24. (U) Cell culture experiments using biochemical, light microscopy and electron micrographic technique.

25. (U) 72 07 - 73 06 A tissue culture laboratory with the capability for synchronizing mammalian cells was established. This laboratory used equipment from the Dermatology Research Division and the Biochemistry Laboratory LAIR, to avoid duplication of major items. A cell line, Chinese Hamster Ovary, was obtained as a gift from Dr. John Harris of the University of California, and is well established under our laboratory culture conditions. Conditioning of flasks and medium has been undertaken in preparation for collection of mitotic cells. Plating efficiencies of asynchronous cells has been determined to be on the order of 75%. Techniques will be devised for improvement of plating efficiency to afford greater reliability in later experiments.

* Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

DERMATOLOGY RESEARCH DIVISION

Program Element 6.21.10.A
Bio-Medical Investigations

Project Number 3A062110A822
Military Internal Medicine

Task Area Number 00

- - - - - - - - - - - - - - -

Project Number 3A062110A831
Tropical Medicine

Task Area Number 00

DERMATOLOGY RESEARCH DIVISION

The Dermatology Research Division studies the interrelated effects alone and in combination of water immersion, heat and humidity, friction, fungal and bacterial infections and insect repellents on man's skin. A multi-disciplinary team approach is used (3 M.D.'s, 3 Ph.D.'s, 5 M.S.'s) in seeking solutions to pressing military dermatological problems. The scientific bases of the research program are the physical and chemical characteristics of the human stratum corneum and cutaneous delayed hypersensitivity.

Mosquito repellents and repellent formulations are being developed and tested on men that resist rub-off, sweat-off, and wash-off substantially longer than those now available.

Volunteers are infected on the forearm with Trichophyton mentagrophytes, the fungus that commonly causes the blistering type of "athletes foot." Their response to the infection is closely studied, particularly the way they develop immunity. Such studies should lead to a vaccine, a better medicine to prevent such a skin disease, and more rapid, accurate, and economical methods to diagnose fungal infections.

Numerous basic scientific studies concern the way substances penetrate and interact with the stratum corneum, to develop antifungal, antibacterial, antifriction, insect repellents, and skin waterproofing substances that will persist in the skin.

One group designs instruments for producing and measuring various degrees of friction under scientifically controlled conditions and develops ways to prevent and treat blisters on the palms and soles of soldiers.

Prickly heat rash (miliaria), its cause and prevention is investigated. Miliaria is produced by putting Saran Wrap patches on a man's back for 2 to 4 days.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6800	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A811	00	155
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) More Effective Topical Repellents Against Malaria Bearing Mosquitoes (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 11	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	1	120.2
	CURRENT 74	1	100.0

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Presidio of San Francisco, CA 94129

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20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Dermatology Research Division

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Spencer, T.S., CPT MSC

TELEPHONE: 415-561-5485

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code) (U) wash-off; (U) sweat-off; (U) human volunteers (U) repellent; (U) mosquito; (U) skin; (U) stratum corneum; (U) polymer formulations

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) In vitro testing methods are being used to determine physical properties of DEET and new repellent formulations to supplement the in vivo screening program. Interaction mechansims between repellents and skin on additives are being studied to predict the evaporation and penetration rates of these formulations in vivo.

24. (U) Polymer formulations and new repellents obtained from Army contractors and industry to be tested in vivo and in vitro.

25. (U) 72 07 - 73 06 A new 4-patch testing technique has been developed permitting better discrimination of repellent formulations with fewer volunteers. A distribution of dry protection time was determined for DEET (standard military repellent) with 32 volunteers. Three new repellents, SRI-7, carboximide, and sulfonamide have been shown to have twice the dry protection time of DEET. Wash resistance of the carboximide and sulfonamide is significantly longer than DEET. In vitro techniques using ^{14}C-deet have been refined to reduce man-hours necessary to carry out repellent evaporation tests. Physical-chemical properties of DEET and successful new repellent formulations have been studied using gravimetric analysis and spectroscopic techniques. Techniques for detection of trace amounts of repellents are being developed using UV absorption and emission spectroscopy. A method for using guinea pigs to screen new repellents is under development.

*Available to contractors upon originator's approval.

More Effective Repellents Against Malaria-Bearing Mosquitoes

Thomas S. Spencer, CPT MSC
W.A. Akers, COL MC

PROBLEM:

Our objective is to develop a broad spectrum, topical preparation which will repel mosquitoes and other arthropod vectors which can transmit malaria, yellow fever, leishmaniasis, and encephalitis and which will persist one or more days resisting removal by excessive evaporation, skin penetration, sweat, water and abrasion. Candidate repellents and formulations for known repellents are obtained from industry and military contractors. These repellents are tested both in vivo and in vitro to determine repellency and physical properties of the repellent molecule. In testing new repellents, experimental variables have been studied to determine their effect on relative protection times in the laboratory.

APPROACH:

Polymer formulations and new repellents are obtained from Army contractors and industry to be tested in vivo and in vitro. These compounds are tested in our laboratories for dry protection time against mosquitoes as compared to the standard military repellent, deet. If superior repellencies and cosmetic acceptability are shown by a new repellent formulation, the repellent is subjected to wash-off tests, sweat-off tests, and tests to determine physical properties in vitro. Candidate repellents showing improved dry protection and sweat-off resistance compared to deet, cosmetic acceptability and no skin irritancy will be considered for future field trials. Once toxicological clearance has been obtained from the USA Environmental Hygiene Agency, field trials will be carried out before recommendations on repellent for general use in the military are made.

If a formulation involves a known repellent such as deet, the screening process is done first in vitro using evaporation and gravimetric properties and energies of activation for the repellent in that particular polymer formulation. Thus, tests using volunteers are not conducted until the formulation has been shown to

be reasonably good as an agent to lenghten the protection time or lower the evaporation rate of the known repellent. In addition, in vitro techniques are used to determine the relative evaporation rates and physical properties of new repellents in order to catalogue these properties and possibly better evaluate new compounds to be tested. Under these circumstances, the mechanisms of interaction of a repellent with a polymer formulation or of a repellent with the skin are studied to determine evaporation rates, penetration rates, and physical-chemical interaction mechanism between the repellent and the stratum corneum.

TESTING METHODS:

A 4-patch repellent testing technique has been developed to economize on the use of volunteer hours. Four different repellents or repellent formulations are applied to four different 7 x 10 cm test patches, two on each ventral forearm of the volunteer. After the test areas have dried for 10 minutes, the individuals are tested hourly in a standard repellent test using two cages of 200 avid female Aedes aegypti mosquitoes each. In a standard test the arm is covered by a plastic sleeve with two 5 x 8 cm cut-outs over the application site. The arm is inserted into one cage of mosquitoes for 3 minutes. If two bites are received in any 3-minute time period or one bite followed by another bite in the succeeding test, the repellent is said to have failed and that site is covered. Volunteers are tested in groups of four, such that the results can be analyzed as a 4 x 4 Latin square statistical design. Two replicates with 8 individuals are completed while the application sequence is rotated to pair each repellent with every other repellent on adjacent patches at least twice and such that each repellent will occupy each of the 4 sites twice.

One site with an application of deet is used as a control, so that all new repellent formulations are compated directly to deet. The advantages of the 4-patch technique are these: (1) each subject acts as his own control, (2) four test subjects can complete a screening test in one day, and (3) tests carried from one day to the next are avoided, thereby minimizing changes in mosquito avidity. No interaction between repellents located on adjacent test sites has been detected in nine months of testing using this method. The absence of interaction between the repellents located on

adjacent sites makes the use of a paired comparison of each new repellent to deet possible, thus we have a better statistical comparison to a standard repellent.

RESULTS:

The 4-patch technique was compared to the 2-patch technique formerly used at LAIR. Repellent applications of deet and 6-12 were made to 8 volunteers. Four volunteers had a normal 2-patch application, one on each ventral forearm. The other 4 volunteers had a 4-patch application with deet and 6-12 applied randomly to 2 of the 4 patches. SRI-6, a test compound, was applied at 2 different concentrations to the remaining 2 patches for the 4-patch technique. On the first day of testing, the individuals underwent the standard mosquito repellent test. On the second day of testing, the groups were reversed; the 2-patch group from the first day becoming the 4-patch group for the second day, and the 4-patch becoming the 2-patch. Two replications, one month apart, were carried out for this experiment. The results shown in Table 1 indicate that there is no significant difference in the discrimination afforded by the 2-patch and the 4-patch techniques. However, using 4 volunteers for one day of testing with the 4-patch technique one is able to test 3 repellent formulations, all compared directly to a standard formulation of deet. Using the 2-patch technique and the same 4 volunteers, discrimination is obtained for only 2 repellent applications. Analysis of site, upper and lower, right and left, and interactions between adjacent sites indicated that there was no significant interaction between any repellent sites in the 4-patch technique. Protection time shown by any given site on an individual volunteer appeared to have no site dependence. Individual variability in comparing a new repellent formulation to deet can be eliminated as an experimental variable by using a paired comparison of each 3 sites to a standard site of deet.

Another experimental variable was studied to determine the effect of human presence on dry protection time afforded by a mosquito repellent. Deet was applied to both arms of 4 volunteers who tested one arm in each of two testing rooms on 2 successive days. During the time in which the volunteers were not undergoing the standard mosquito repellent test, 4 other volunteers were harrassing the mosquitoes, making the mosquitoes aware of a continued human presence by inserting arms

into the cage, putting hands next to the mosquito cage, or blowing periodically into the cage. Towards the end of the testing period at 6 to 8 hours for the standard deet applications, the cages on both days which had been harrassed on a continual basis showed a much markedly lower mosquito activity and mosquito avidity. Apparently, continued human presence can lower a mosquito's activity in a testing situation.

In a test to determine the effect of the ambient temperature in which the volunteer is working on the dry protection time afforded by a repellent, two groups of four volunteers were put in two rooms, one at a high temperature, one at a moderate temperature. No significant increase or decrease in protection times was noted between a room at 23° and the room at 28°C.

An arm guard was tested to determine the effect of abrasion on repellency time. The arm guard showed that in an overnight situation, where repellents are applied in the evening and tested the next morning, an arm guard significantly increases the protection time over an unprotected site. This information will be useful in testing the longer lasting repellents with which LAIR is working at present.

PROFILE OF DRY PROTECTION TIME OF HUMAN VOLUNTEERS: To determine a mean repellency time for our volunteers, the entire contingent of volunteers and associated personnel working with the mosquito repellent program were tested to determine the average dry protection time for deet using a patch the size used in the 4-patch method. Repellent was applied to 32 volunteers in groups of 5 on the test day. The repellency times and standard repellent test were done in 6 cages, such that each volunteer tested in each cage on a randomly selected basis. Only 5 of the 6 cages were used during any given test period. The resulting dry protection time show that our volunteer population has a mean deet protection time for this given test of 6.8 hours (SD + 1.9 hr). The distribution is close to a normal distribution with a standard error of the mean of 0.3 hours. The profile obtained in this test with 32 volunteers is shown in Graph 1. Results of this test will be used in evaluating the consistency of experimental results in future tests using human volunteers. In addition, this test has enabled us to place volunteers in 3 categories: more attractive, average

attractancy, and less attractive than average. This information will aid us in determining which volunteers will be used for experiments in which a high attractancy or a low attractancy is desirable. Currently, work is being done to correlate the background and physiological data on these volunteers with their position in the mean normal distribution of dry protection times afforded by deet.

Several new repellents and additives for the present standard military repellent, deet, have been tested during the present fiscal year. The results shown in Table 2 indicate dry protection times as compared to deet which is the standard repellent at present. The values reported in Table 2 are reported as mean differences between a paired comparison of a repellent formulation to the dry protection time of deet. In each case the average protection time of deet among 7 individuals is included in Table 2. The 3 most exciting repellents tested this year are the Russian sulfonamide, n-butane-N-hexamethylene-imine sulfonamide, N-N-hexamethylene-imine carboximide, and SRI-7, a glycol formulation submitted by SRI. In terms of dry protection time, these 3 compounds (Table 2) showed protection times twice that of deet. The average mean differences among an individual volunteer, between deet and the other 3 sites, and the standard deviation of the mean differences are shown also on the graph, indicating that the difference is consistent among volunteers. Batches of these compounds were synthesized by SRI for toxicological testing at USAEHA. In addition, 1/g kg was synthesized for further testing at LAIR and possible field trials this summer at Camp Lejeune, N.C. A fourth compound, SRI-6, will be tested at Camp Lejeune, since it costs the least. SRI-6 compound on the average has better protection time than deet (Table 2). However, note that SRI-6 works extremely well for some individuals and only as well as deet for other individuals. This phenomenon is believed to result from a high minimum inhibitory concentration for mosquito repellency for SRI-6, such that SRI-6 works very well at 2 mg/in^2 but not nearly so well as deet at lower concentrations. Field applications of this repellent are well above the 2 mg/in^2 normally used in our testing procedures; therefore, we believe that SRI-6 could be an effective repellent in field use.

Two additives, LTH and VLN, were tested in various formulations with deet and other new repellents. LTH, submitted by Boyle American Midway, Inc., was first tried with meta-deet forming a liquid which could be put in an aerosol. The effective dry protection time (Table 2) was not significantly longer than that of deet control in a 2:1 ratio, deet:LTH. Wash results reported in Table 3 indicated similarly disappointing results. The mixture of LTH and para-deet formed a solid grease-like mixture. These findings were reported to the manufacturer who produced a refined mixture of LTH in a stick repellent, which we tested. This stick repellent, although cosmetically acceptable, did not increase the dry protection time of the deet control. On the other hand, the VLN additive formulations in ratios of 1:2, 1:1 and 2:1 mixtures with deet showed that a 2:1 mixture of deet to the VLN additive doubled the dry protection time of deet. VLN additive was tested also with SRI-6 and is being tested using the sulfonamide and the carboximide to determine if VLN will enhance the repellency times of these repellents also.

WASH PROTECTION TIME:

Wash protection tests were developed using the 4-patch technique and the 4 best known mosquito repellents, as reported last year. The results show that the 4-patch technique is effective in determining differences in wash resistance among repellents. A study of the effect of flow rates and water temperatures for wash-off studies indicated that wash-off times were minimally dependent on flow rates varying from 50 to 200 ml over a 10 second interval (Graph II). On the other hand, increasing the water from 27°C to 37°C seemed to have a significant effect. The normal skin temperature, approximately 32.5°C, was chosen as the wash temperature of choice to prevent changes in the skin temperature of the arm being tested for mosquito repellency. A flow rate of 100 ml in a 10 second period was chosen with the wash times being reported in cumulative total number of seconds wash time prior to a failure of a repellent site. Table 3 shows the wash protection results. The most significant portion of Table 3 is the comparison of the two Russian repellents to deet. The sulfonamide and the carboximide both exhibited superior wash resistance to that of a deet control. A mixture of the sulfonamide plus additive VLN and deet plus additive VLN showed slightly

longer wash protection times in a second wash test; however, the results are not statistically significant at the 5% level.

Other additives tested in wash protection tests indicate that LTH does not significantly increase the wash protection time of deet and that the SRI-6 mixed with VLN does not have significantly longer protection time than deet itself. However, addition of VLN to SRI-6 does make the SRI-6 wash protection time comparable to deet.

ANIMAL MODEL FOR MOSQUITO REPELLENTS:

In order to develop an effective animal model for mosquito repellent screening tests, we are currently working with Moen-Chase guinea pigs and Mexican hairless dogs. A 5 x 5 cm area on the back of the guinea pig is clipped and repellent solution is applied to this area. A foam rubber pad, 1/2" thick, is then placed around the area with a cut-out in the center. The guinea pig is then inserted into a sock such that the guinea pig, believing that he is hiding, will remain quiet during the course of the experiment. The standard repellent test is carried out at hously intervals by exposing the repellent area of the guinea pig through a trap door in the bottom of a standard mosquito repellent cage. Tests comparing volunteers to guinea pigs (Table 4) have yielded results indicating that guinea pigs obtain longer dry protection times from deet than humans. The end result of these tests will be a profile or statistical average of several guinea pigs compared to a profile or statistical average among several volunteers using the same concentration testing with the same mosquitoes on the same day. The results will give us data for the potential use of an animal model for repellent screening.

FIELD TRIALS OF MOSQUITO REPELLENTS:

We visited the California Mosquito Control Laboratories of the University of California near Fresno to test mosquito repellents against different species of mosquitoes than the Aedes aegypti used in our laboratory testing. Tests using Culex pipiensand Aedes taeniorhynchus compared to Aedes aegypti which were transported from our laboratory to Fresno indicated that the Aedes aegypti were much more avid than the other species tested. These results were obtained

using Russian sulfonamide compound, SRI-6, and deet, the standard military compound. Further field tests against species native to California are being anticipated for this summer.

A field test in August will be conducted at Camp Lejeune, N.C., where an area exists in which pesticides are not used and mosquitoes are allowed to breed freely. Working with Navy entomologists at the US Navy Field Medical Research Laboratory, Camp Lejeune, we plan to test the three most successful repellents tested in this fiscal year versus the standard military repellent, deet, in order to determine the efficacy of these repellents under field conditions. Volunteers from LAIR will be used in these trials to compare the field trials to our own laboratory tests. The inherent repellency or attractancy of these volunteers has been well documented in our mosquito repellent studies at LAIR.

IN VITRO STUDIES:

To evaluate the effectiveness of new repellents and repellent formulations, techniques have been developed to study the physical properties of new repellents and repellent formulations, including rates of evaporation of the repellents, minimum inhibitory dosages, boiling point, energies of activation for evaporation from surfaces (i.e., the skin), and heats of vaporization for various repellents. By making these determinations for known repellents with well documented repellency times, we can compare the physical properties of a repellent like deet with the physical properties of a new repellent in order to find factors which might affect evaporation rates or penetration rates of the new repellents. In addition, we can investigate the mechanism of interaction of the new repellent formulations with the skin or additives which might possibly be used with the new repellents. Two techniques are currently being used in these studies, (1) an evaporation technique using an apparatus (Evap) which was described in previous annual reports and which has been refined this year, and (2) a gravimetric technique by which the rate of evaporation of a repellent can be determined from a liquid or from a surface. Preliminary results in this sort of experiment showed that 612 has twice the evaporation rate of deet at 30°C and SRI-6 has an evaporation rate about 25% of deet. The energy of activation of all the compounds is between

16-20 k cal/mole. This determination is made by a plot of the natural log of the evaporation rate versus the reciprocal of the absolute temperature at which the evaporation rate is determined.

Results from the Evap apparatus using ^{14}C-deet showed that deet evaporates at rates on the order of 50 ug/cm^2/hour. A 1:1 mixture of deet:VLN has an evaporation rate on the order of 20 ug/cm^2/hour and mixtures of deet:VLN in a 1:2 ratio has evaporation rates on the order of 10 ug/cm^2/hour. These results are in agreement with the experimental results determined by in vivo testing with the deet:VLN mixtures. Hence, the utility of the apparatus for future screening has been shown. Refinements which have been made during the present fiscal year have been a new timing system such that samples may be taken over longer periods of time and fewer samples have to be collected for a given experimental run. Also, new valves and trapping systems have been installed to prevent mishap and to add safety in the use of radioactive or radiolabeled materials. All of the glassware used in the apparatus is now siliconized to avoid time lags in sample evaporation. As a final phase of the in vitro mosquito repellent program, interactions between mosquito repellents and additives have been studied spectroscopically to determine the molecular interaction between the repellent and the additive. Preliminary results show (1) that we can detect when an interaction is occurring between a repellent and an additive and (2) that we can determine the functional group responsible for the interaction in some cases. Further work is being done along these lines to quantitate the results which have been observed in the preliminary experiments.

Table 1.

Comparison of 4-Patch and 2-Patch Testing Methods

Repellent	Dry Protection Time[1] (hours) 4-Patch	2-Patch
Deet	5.9	6.8
612	3.1	3.0
SRI-6	6.2	
SRI-6[2]	3.2	

[1]Application rate 0.31 mg/cm^2

[2]Application rate 0.61 mg/cm^2

Table 2.

Repellent Dry Protection Times

Repellent	Additive	Ratio	Protection Time (hours) Deet Control[2] (Application Rate)	Test Repellent
612				
Sulfonamide			7.6	14.1
Carboxamide			7.6	16.2
SRI-7			7.6	16.8
SRI-6			5.8	6.2
Deet	LTH	3:1	5.25	6.5
Deet	LTH	4:1	5.25	6.0
p-Deet[4]	LTH	2:1	3.4[3]	0.5
p-Deet[4]	LTH	1:2	3.4[3]	0.5
Deet	VLN	1:1	3.4	8.3
Deet	VLN		3.4	6.5
VLN			3.4	0.9

[1]Applied at 0.31 mg/cm^2

[2]m-Deet control except where noted

[3]p-Deet control

[4]Applied at 0.155 mg/cm^2

Table 3.

Wash Resistance of Repellents

Repellent*	Additive	Ratio	Wash Protection Time (sec) Deet Control	Repellent Formulation
Carboxamide			53.75	75.0
Sulfonamide			53.75	86.25
Deet	LTH	2:1	53.75	42.50
Deet	VLN	1:2	31.3	43.8
Sulfonamide	VLN	1:1	31.3	40.0
SRI-6	VLN	1:1	31.3	32.5

*Applied at 0.31 mg/cm^2

Table 4.

Dry Protection Times
Guinea Pigs Compared to Volunteers

	Repellent Concentration	Dry Protection Time (hr)	Std. Dev.
Guinea Pigs (5)	0.08 mg/cm^2	2.5	± 0.71
Volunteers (5)	0.08 mg/cm^2	1.3	± 0.45
Volunteers (5)	0.155 mg/cm^2	3.1	± 0.89

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6907	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A0611040S11	00	15
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Studies on Blistering Produced by Mechanical, Thermal and Chemical Agents (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 03	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 73	0	4.1
FISCAL YEAR CURRENT 74	0.5	20

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

ADDRESS:

RESPONSIBLE INDIVIDUAL

NAME: Akers, W.A., COL MC

TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Dermatology Research Division

ADDRESS: Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Akers, W.A., COL MC

TELEPHONE: 415-561-5181

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) friction; (U) blisters; (U) prevention; (U) feet; (U) skin; (U) blistering machine; (U) human volunteers

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To study the epidermis, its chemical nature and physical properties when subjected to frictional injury in order to find ways to raise the threshold of susceptibility to skin frictional injury and reduce the disability and sequelae produced from blisters in soldiers on long marches.

24. (U) Experimental friction blisters by linear and twist rubbing will be used as a model of studying the prevention and treatment of friction blisters.

25. (U) 72 07 - 73 06 - This work unit was held in an inactive state for one year. During this fiscal year, a detachable strain gauge is under development to measure and permit calibration of torque at the tip of our friction blister rubbing machine. Next year work will begin on frictional aspects against man's skin of design and materials for the proposed new combat boot.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6814	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	H. Terminate	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	159
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Studies on Blistering Produced by Mechanical, Thermal, and Chemical Agents: Kinetics of Blister Fluid Proteins (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 002300 - Biochemistry; 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 12	CONT	DA	C. In-house

17. CONTRACT/GRANT:
a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR 73	0	1.6
CURRENT YEAR 74	0	0

19. RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Institute of Research
ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL
NAME: Akers, W.A., COL MC
TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION
NAME: Letterman Army Institute of Research
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Schmid, P., Ph.D.
TELEPHONE: 415-561-5485
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE: DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) biochemistry; (U) blister fluid; (U) albumin; (U) fibrinogen; (U) immunoglobulins

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To study the reconstitution of the skin in terms of the barrier function following production of friction, thermal, and chemical blisters by studying the change in concentration of selective proteins with time after blistering. To characterize proteins in vesiculobullous lesions and pustules due to various diseases.

24. (U) Friction, thermal and chemical blisters will be produced on volunteers. Fluid from experimental blisters and lesions will be collected and selected proteins measured.

25. (U) 72 07 - 73 06 Work unit is terminated because of manpower limitations.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6815	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. GROUP MBR'S	9a. SPECIFIC DATA/CONTRACTOR ACCESS	9b. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	160
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with security classification code)

(U) Clinical Evaluation of Alpha-Cyanoacrylates and Treatment of Friction Blisters(05

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine; 012600 - Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 05	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	73	.1	2.1
CURRENT	74	.1	12.0

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
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ADDRESS:

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TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
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ADDRESS: Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Akers, W.A., COL MC

TELEPHONE: 415-561-5181

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21. GENERAL USE

22. KEYWORDS (Procede EACH with security classification code)

(U) isoamyl-cyanoacrylate; (U) blister therapy; (U) topical

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) To develop a method of therapy for friction blisters of the feet which can be used by the soldier in the field to reduce ineffectiveness, pain, infection, and to promote healing.

24. (U) Clinical evaluation of the cutaneous reactivity and sensory response to the topical application of cyanoacrylatè homologues on experimental friction blister bases.

25. (U) 72 07 - 73 06 In collaboration with the Division of Preventive Medicine, Walter Reed Army Institute of Research, and the Department of Dermatology, University of Miami, a 1Ø week fieldtrial was conducted among U.S. Army Ranger students in the mountains of Georgia and the swamps of Florida. Two of the platoons had intensive prophylactic skin care, supervised by a scientist, including the use of isoamyl-cyanoacrylate on denuded friction blister bases. Cultures for bacteria and fungi were done in the field. Preliminary results indicate the cyanoacrylate therapy was enthusiastically accepted by the soldiers. One treated site became infected. Microbiological analysis is incomplete. Future field trials are planned.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6911	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	H, Terminate	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A061102AB72	00	161
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Biosynthesis of Proteins and Lipids in Human Skin with Particular Emphasis on Prickly Heat (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 002300 - Biochemistry; 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 06	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PREVIOUS 73	0	2.7
FISCAL YEAR CURRENT 74	0	0

19. RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Institute of Research
ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL
NAME: Akers, W.A., COL MC
TELEPHONE: 415-561-5181

21. GENERAL USE

20. PERFORMING ORGANIZATION
NAME: Letterman Army Institute of Research
ADDRESS: Dermatology Research Division
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Schmid, P., Ph. D.
TELEPHONE: 415-561-5485
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) biochemistry; (U) prickly heat; (U) protein synthesis; (U) lipid synthesis; (U) skin; (U) human volunteers

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To determine the role of heat stress on protein and lipid synthesis in prickly heat resistant and prone individuals. To develop therapeutic counter measures against prickly heat.

24. (U) To develop microtechnology for determination of suluble proteins and acids of hydrolyzed proteins. To measure protein and lipid synthesis in skin scrapings in prickly heat resistant and prone individuals.

25. (U) 72 07 - 73 06 Work unit is terminated because of manpower limitations.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6912	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	162
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Studies on the Effects of Heat & Humidity Upon the Human Skin with Particular Emphasis on Prickly Heat & Consequent Disabling Dermatoses(05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 08	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR 73	1	1.6
CURRENT 74	1	54

19. RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Institute of Research
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RESPONSIBLE INDIVIDUAL
NAME: Akers, W.A., COL MC
TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION
NAME: Letterman Army Institute of Research
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Akers, W.A., COL MC
TELEPHONE: 415-561-5181
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Schmid, P. Ph.D.
NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede each with Security Classification Code): (U) miliaria; (U) prickly heat; (U) rash; (U)sweating; (U) heat retention; (U) stratum corneum; (U) skin; (U) heat fatigue

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To find the causal mechanism, means of prevention, treatment, and the interrelationship of prickly heat rash (miliaria) to disturbances in sweating due to heat retention (hypohidrosis) and heat fatugue in soldiers.

24. (U) Volunteers prone or resistant to experimental prickly heat and subsequent decrements in sweating are characterized regarding skin bacteria, skin surface fats, skin pH, and hydration dynamics of the horny cell layer.

25. (U) 72 07 - 73 06 Of 26 lanolin fractions, 6 were tested and compared to lanolin in their effectiveness to prevent miliaria. Two of the fractions appear superior to lanolin.

Studies on the Effects of Heat and Humidity Upon the Human Skin with Particular Emphasis on Prickly Heat

W.A. Akers, COL MC
Peter Schmid, Ph.D.

PROBLEM:

In World War II, investigators observed that heat cramps and heat exhaustion were associated with transient reductions in sweating. Subsequently, a laboratory model for the production of experimental miliaria (prickly heat rash), using occlusive polyethylene wraps over a 72 hour period, was developed at the Letterman Army Institute of Research (LAIR).

APPROACH:

Prickly heat serves as a prototype to investigate cutaneous diseases associated with assaults of heat and humidity upon the stratum corneum. To study the causal mechanisms, means of prevention and treatment of these disorders, volunteers prone or resistant to experimental prickly heat and subsequent decrements in sweating are characterized regarding skin bacteria, skin surface fats, skin pH, and hydration dynamics of the horny cell layer.

RESULTS AND DISCUSSION:

Previous studies in this laboratory indicate that anhydrous lanolin retards or prevents experimental miliaria produced with the small patch occlusion technique. Since lanolin is one of the few agents which prevents experimental miliaria it was of interest to test commercially available subfractions of lanolin and lanolin derivatives. Based on evaluation of physical, chemical and biochemical data, 6 of 26 compounds were chosen for testing in a randomized multiple patch experiment on 8 volunteers. Eight sites of 2.5" x 4" were marked with dihydroxyacetone on each volunteer and sweat imprints of each site made prior to the start of the experiment as described below.

At the start of the experiment 6 lanolin fractions and anhydrous lanolin were applied in a random fashion at a concentration of 4 mg/cm^2. Patches were applied as described by Sulzberger and Harris (Arch Derm, 105: 845, 1972). Patches were inspected at 2, 30, 44 and 72

hours for leaks or tears, the observations recorded, and, if necessary, patches repaired. Based on the recorded observations a reliability factor was defined and the factor calculated for each site of each volunteer. At the end of 72 hours, patches were removed. Any remaining agent was removed with a cotton pad. The volunteer then exercised at 33°C and 42% relative humidity for a period of about 20 minutes until generalized profuse sweating was observed.

The six lanolin fractions tested were: acetol, ethoxyol, lanacet, isopropyl lanoate, trisolan and lawfrax.

Miliaria was graded at room temperature on a scale of 1 to 4 with a reading of 1 indicating no miliaria and 4 indicating extensive miliaria. Photographs on appropriate sites were also taken. Following an additional heat stress of about 10 minutes, under conditions indicated above, sweat imprints were taken. Preliminary analysis of the data indicates that 2 of the lanolin fractions appear to be superior to lanolin in preventing miliaria as measured by miliaria grading and sweat gland function. The two fractions appear superior to lanolin in regard to cosmetic acceptability also.

FUTURE PLANS:

No full-time investigator is available next fiscal year for this project, so few experiments will be made.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6912	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61110A	3A062110A822	00	163
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Composition of Lipids (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 73	1	42.4
CURRENT FISCAL YEAR 74	1	35.0

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

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RESPONSIBLE INDIVIDUAL

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TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
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ADDRESS:
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Schmid, P., Ph. D.

TELEPHONE: 415-561-5485

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) fungal infection; (U) water immersion (U) soldiers (U)biochemistry; (U) lipids; (U) fatty acids; (U) glycerides; (U) water immersion;

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To determine and compare the composition and quantities of skin lipids in soldiers under normal and adverse conditions, such as before, during and after fungal infection, before and after prolonged water exposure, tropical acne patients, etc., to test more effective therapeutic agents for the treatment or prevention of these skin diseases.

24. (U) To develop new techniques to determine the composition and structure of minute quantities of lipids. Apply the evolved techniques to study surface skin lipids in conditions listed under item 23.

25. (U) 72 07 - 73 06 We have developed a new infrared spectroscopic method to determine crude lipid extracts which does not have the inherent drawbacks of the now standard gravimetric technique. The method has been applied in a pilot study on experimental fungus infections in the skin of man in conjunction with another LAIR work unit. Additional adsorbents for high pressure liquid chromatographic separations of complex skin lipids were tested to improve reproducibility and speed of the separations.

Composition of Lipids

Peter Schmid, Ph.D.

PROBLEM:

Fungal infections of the feet, groin and trunk are among the most common military medical problems. Under comparable nutritional and climatic conditions, a certain type of soldier will suffer from chronic infection of T. mentagrophytes, whereas another type is not infected. The reasons for this difference in susceptibility are not known at present. It is possible that for genetic or other reasons the quantity and/or quality of epidermal and/or sebaceous lipids in the foot region may be different in the two populations. If this were the case, the acquired knowledge of the differences in skin lipids might be used to design and test agents to prevent fungal infections in soldiers.

APPROACH:

A series of 2 experiments were performed to gain knowledge on skin irritancy, amount of crude lipid and composition of lipid on fully and partially immersed feet of subjects.

EXPERIMENT #1:

In these experiments, healthy subjects with no or minimal fungal infection were used. Differentiation between epidermal lipid amount and composition on the sole and sebaceous lipid (by difference) from the toe is attempted. Because of limited knowledge on skin irritancy of toluene ethanol, three different extraction methods were used. A first attempt was made to compare lipid extractions and composition of lipids with toluene-ethanol from: (a) feet immersed above the ankle (full foot immersion), (b) the soles of feet immersed (top of foot and ankles not immersed), and (c) toe portion of feet immersed (ankle and heel not immersed).

METHOD:

Key dimensions of the foot of the subjects were measured 24 hours before collection of lipids. The foot was then washed in a Triton-X solution, rinsed with distilled

water and dried. The subject's foot was covered with a pair of clean army-regulation socks and the subject instructed not to take the socks off until extraction of lipids.

At collection time, socks were removed and the foot immersed above the ankle, the sole only or the toe portion of the foot only, respectively. During the 3 minute immersion time, subjective responses of the volunteer were recorded. In addition, before and after extraction, color photos were taken. Solvent was removed in vacuo and analyzed by the gravimetric and infrared method.

RESULTS:

a. Skin irritancy: Of 18 feet fully or partially immersed in the organic solvent, 17 feet showed no erythema or an erythema of short duration. One subject developed a vesicular eruption at the end of about 3 hours following immersion which lasted for several days. Subsequent patch testing with routine test substances and agents used in the experiments indicated no reaction. We attribute the eruption to a combination of defattening of the skin, friction and exercise unrelated to the experimental procedure. No hypopigmentation or hyperpigmentation was observed on any subject's feet contrary to previous experiments with chloroform-methanol mixtures.

b. Foot area measurements: Because of the fairly large variation in foot size between subjects, calculations of the area from which lipids were extracted was made. Calculations are based on simplifying assumptions, approximate areas are as detailed in Table I. The calculated mean value and the range is indicated.

c. Lipid determinations: The gravimetric procedure as well as measurements with the quantitative infrared spectroscopic method were made. In Table II the amount of crude lipid per area is listed for sole-, toe- and ankle-immersion. The results suggest the amount of crude lipids extracted per area from the sole to be less than the amount extracted from the toe. Since the lipids extracted from the sole are primarily of epidermal origin and since very few, if any, sebaceous glands can be identified, it is reasonable to expect a lower concentration on the sole than on the toe. However, verification on area measurements

by an independent method is needed. Purification of crude lipids by the sephadex method (developed in this laboratory) needs to be performed as well as further separation and quantitation of subfractions of lipids.

Progress on the lipid studies is hampered because of the high turnover rate of laboratory technicians who need extensive training before becoming productive members of the team.

FUTURE PLANS:

Area measurements by an independent method needs to be done to verify assumptions made in the present analysis. Corrections to the model calculations may have to be made if necessary. Continue analysis of samples already obtained. Perform additional experiments with volunteers, if needed, to firm up results.

EXPERIMENT #2:

In this experiment, subjects classified as "fungal virgins" and subjects with active fungal infections were used. The lipids from the toe portion of one foot and the sole portion of the other foot were to be compared between "fungal virgins" and subjects with fungal infections.

METHOD:

Methods described in Experiment #1 were used in this study.

RESULTS:

a. Skin irritancy: Of 20 feet immersed in the organic solvent, 19 feet showed no or short transient erythema. One foot showed erythema lasting for several hours. In no case was there any hypo or hyperpigmentation observed.

b. Foot area measurement: Using the same type of measurements and assumptions as in Experiment #1, toe area measurements are given in Table III for the virgin group as well as the active fungal infection group. Means and plus and minus 95% confidence limits are given. The data suggest that the area of foot sampled for the virgin and experimentally infected

group is not statistically different, i.e., the foot size of the two groups is comparable. However, caution is required as indicated in Experiment #1.

c. Lipid determinations: These determinations were made as described in Experiment #1, part c. Table IV lists the mean value and range in microgram per cm^2 for total toe lipid, epidermal toe lipid and sebaceous toe lipid for the virgin group and infected group. It appears that the epidermal lipid of the toe, i.e., the contribution from the sole part of the toe, are not very different for the virgin group and the infected group. The table furthermore suggests that there appears to be a difference in the concentration of sebaceous lipid, i.e., the upper side of the foot of the virgin group contains some 14% less lipid per area than the infected group. Sample size is, however, small and accuracy of area measurements must be verified as pointed out in Experiment #1. For both groups the upper part of the toe portion of the foot contains significantly more lipid per area than the sole portion of the toe portion of the foot.

FUTURE PLANS:

Difficult and complex problems in relation to meaningful measurement of skin lipid of military and general importance have been overcome in the last few years. The results are beginning to indicate differences where previously no differences could be expected. With the new methods such questions as why groups of people are infected by fungi while others are not can now be asked and meaningful answers can be expected.

Table I. Area of Foot Immersed

	Area cm^2	
	Mean	Range
Sole	428	370 - 540
Toe	320	300 - 330
Ankle	600	420 - 660

Table II. Concentration of Lipid on Skin Surface

	$\mu g/cm^2$	
	Mean	Range
Sole	99	68 - 140
Toe	127	117 - 143
Ankle	100	63 - 155

Table III. Toe Area for Experimental Subjects

Type of Subjects	-95% C.L.	Mean	+95% C.L.
"Virgins"	266.8	284.9	302.9
Fungal Infected	260.7	287.9	315.2

Table IV. Toe-Lipid Level - ug/cm^2 for Two Groups of Subjects

	Microgram/cm^2	
	Virgin	Infected
Total Lipid		
Mean	103.3	107.4
Range	88.6 - 114.0	92.2 - 123.2
Epidermal Lipid		
Mean	75.5	77.0
Range	65.9 - 85.3	70.2 - 85.5
Sebaceous Lipid		
Mean	144.5	164.2
Range	98.8 - 180.0	109.5 - 196.3

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6913	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62 10A	3A062110A822	00	144
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Physical, Chemical Characteristics of Human Stratum Corneum (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 07	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 73	1	7.7
FISCAL YEAR CURRENT 74	2	55

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NAME: Trager, G., CPT MC
NAME:

21. GENERAL USE
DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) stratum corneum; (U) absorption; (U)permeability; (U) water; (U)water vapor; (U) chemicals; (U)persistenc; (U)skin;(U)human volunteers

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To define the physical-chemical characteristics of the stratum corneum (SC) and its interaction with water, chemicals, UV radiation and environment. These characteristics are fundamental to the etiology of several dermatological disorders caused by exposure of the soldiers' skin to the environment and the behavior of topical preparations.

24. (U) SC will be harvested using different harvest and workup techniques. Techniques will be developed to assay the denaturing effects of harvest method, water, UV radiation and chemical exposure in addition to environmental conditions on SC. SC from individuals prone vs. individuals resistant to disabling dermatoses will be evaluated before & after experimental induction of such disabling conditions. The efficacy of protective formulations in preventing hydration, denaturation and UV denaturation of SC will be studied.

25. (U) 72 07 - 73 06 Study suspended FY71 due to REFRAD of principal investigator. The apparatus for gravimetric assay of hydration in human environment has been redesigned to permit better control of temperature and humidity during the experiment. New recording devices permit better discrimination of weight gain and more stable signals from the Cahn Electrobalance RG. The new apparatus produces results similar to those obtained previously. Water gain of a SC sample varies inversely with the sq root of time as an exponential log function indicating that the rate of hydration of the SC is a function of the degree of hydration of the SC. A permeability cell has been set up to study the rate of penetration of some topical applications and mosquito repellent through SC samples obtained by various harvest techniques. Radko-labelled compounds will be used in the chamber.

Physical, Chemical Characteristics of Human Stratum Corneum

Thomas S. Spencer, CPT MSC
George Trager, CPT MC

PROBLEM:

The problem concerned with studies of the stratum corneum is to define the physical-chemical characteristics of the stratum corneum proteins, and the interactions of the stratum corneum with water, chemicals, ultraviolet radiation and the environment. Since these characteristics are fundamental to the etiology of several dermatological disorders caused by exposure of the soldier's skin to the environment and the behavior of topical preparations, the physical characteristics of the stratum corneum are of prime consideration in protecting a soldier from his environment. Definition of the chemical properties will aid in protecting the tissues underlying the stratum corneum from various elements assaulting the integrity of the skin.

APPROACH:

Stratum corneum (SC) will be harvested using different harvest and work-up techniques, to include cantharidin blister tops and cadaver stratum corneum. Techniques have been developed to assay the denaturing effects of harvest method, water, ultraviolet radiation, chemical exposure and environmental conditions on SC. SC from individuals prone versus individuals resistant to disabling military dermatoses will be evaluated before and after experimental induction of such disabling conditions. The efficacy of protective formulations in preventing hydration, denaturation and ultraviolet degradation of SC will be studied. In addition, the effect of molecular compounds and protective formulations will be studied relative to their active sites within the stratum corneum membrane. The latter aspect will include studies of permeability and mechanisms of interaction between topical applications and the stratum corneum itself.

PROGRESS:

The apparatus for gravimetric assay of hydration in a human environment has been redesigned to permit better

control of temperature and humidity variations during the experiment. In addition, new recording devices permit better discrimination of weight gain and more stable signals from the Cahn Electrobalance RG, mentioned in previous annual reports. Samples currently being run show that the new apparatus does indeed produce results similar to the results produced showing that water gain of a stratum corneum sample varies inversely with the square root of time in a natural log relationship, indicating that the rate of hydration of the stratum corneum is a function of the degree of hydration of the stratum corneum. Accurate temperature control systems have enabled us to determine the effect of temperature on the degree of hydration at a given relative humidity. Work is being continued in this area.

A permeability cell has been set-up to study rates of penetration of some topical applications in mosquito repellents through stratum corneum samples obtained by various harvest techniques. Radiolabelled compounds presently are being used in the chamber. By use of this chamber we hope to determine simultaneous evaporation and penetration rates of a given application from the surface and through the stratum corneum sample. When the system is completed, we should be able to determine the main routes or mechanisms of loss of a topical application or mosquito repellent from the stratum corneum. By comparing the relative rates of penetration and evaporation of applications of varying molecular structure, we hope to determine the mechanisms responsible for different permeabilities and evaporation rates associated with given molecular systems. This will aid in determination of the physical characteristics of the membrane, stratum corneum, which protects the body from assaults by the environment.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6915	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	167
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Skin Diseases Among Soldiers (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 10	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	1	6.1
	CURRENT 74	1	24

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NAME:
DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code) (U) occupation; (U) environment; (U) patients; (U) diagnosis (U) epidemiology, (U) incidence; (U) prevalence; (U) frequency; (U) climate; (U) skin

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To ascertain the clinical frequency by which all skin diseases are seen, both in dependents and active duty soldiers, and to determine the relationship between the diseases seen and environmental factors.

24. (U) A computer supported data collection in which each dermatology clinic visit at Walter Reed, Brooke, Fitzsimons and Letterman Army Medical Centers is indexed and subsequently cross-correlated with environmental data. The study will be conducted over a 2-year perior and will include over 120,000 patient visits.

25. (U) 72 07 - 73 06 The four hospital computer supported information system has been operative for nine months, and between 45,ØØØ and 5Ø,ØØØ dermatology outpatient visits have been processed.

*Available to contractors upon originator's approval.

Skin Diseases Among Soldiers

Henry E. Jones, LTC, MC
Margaret L. Barba, DAC

PROBLEM:

Little information is available on what skin diseases are seen frequently in civilian and military dermatology clinics, and almost nothing is known about the actual incidence and prevalence of skin diseases in a population.

While most clinicians know that the environment influences skin disease, the relative importance it may hold and the relationship between specific environmental factors and causation or aggravation of any one disease has not been studied in detail. The primary purpose of this study is to ascertain the clinical frequency by which all skin diseases are seen, both in dependents and active duty soldiers, and to see if there is any relationship between the diseases seen and environmental factors. It might be possible to predict epidemics of skin disease from this type of study.

APPROACH:

A pilot study was completed utilizing the clinics at William Geaumont General Hospital and the 95th Evacuation Hospital in Vietnam. The experience gained from the pilot study indicated that a large scale study was feasible and desirable. The dermatology clinics at Walter Reed, Brooke, Fitzsimons and Letterman Army Medical Centers began a computer supported data collection system on 1 July 1972, in which each clinic visit is indexed and subsequently the data bank will be cross-correlated with environmental data. The study will be conducted over a 2 year period and over 120,000 patient visits will be included in the data bank.

RESULTS:

The pilot study involving dermatology clinics at William Beaumont General Hospital and the 95th Evac Hospital in Vietnam has been completed and a publication prepared and submitted to the Archives of

Environmental Health. The pilot study determined that the most significant correlation between the environment and skin diseases occurs with those diseases involving the superficial layers of the skin (stratum corneum) and of the environmental factors considered, the water content of the air expressed as the relative humidity or absolute humidity shows the closest correlation with cause or aggrevation of these skin diseases. Photosensitive diseases were more frequent when the number of hours of terestial sunlight was at its peak, and it should be noted that this is much greater at an elevated altitude than near sea level, but appears to be unrelated to the latitude of the areas studied.

The four hospital computer supported information system has been operative for nine months, and between 45,000 and 50,000 dermatology outpatient visits have been processed. Most of the problems encountered initially in processing this number of cards, computer turnaround time, obtaining patient and doctor cooperation, keypunching and computer analysis problems have been eliminated. The four hospitals participating in the data collection system receive a monthly report of the data processed for use in managing the clinic. They also receive a quarterly report of the data processed, which gives a breakdown by doctor as to the number of patients and the types of diseases seen, which is of assistance in evaluating the progress of a doctor in the training programs.

The computer supported data collection system lends itself to indexing by any of several parameters, including diagnosis, and this has provided support to clinical studies at Letterman Army Medical Center where it was desirable to obtain a listing of all patients seen during the past several months for the diagnosis of dermatophytic fungal infections and herpes virus infections. It is anticipated that the data bank will be more useful in the area of clinical research in the coming months and should become a valuable clinical research tool.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6913	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	168
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) The Effects of Prolonged Water Exposure on Human Skin (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREA

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 04	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PREVIOUS 73	1	5.7
FISCAL YEAR CURRENT 74	1	50

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21. GENERAL USE

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ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

22. KEYWORDS (Precede each with Security Classification Code) (U) warm water immersion foot; (U) paddy foot; (U) human volunteers; (U) skin; (U) skin damage; (U) water composition

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To define the clinical, histopathological, physical, biochemical, and immunological changes that result from prolonged exposure of skin to water. To guide the development of specific and effective prophylactic and therapeutic measures.

24. (U) Study the pathologic changes induced by prolonged water exposure using small localized areas on human skin. Determine and compare specific evolutionary changes in clinical, histological, chemical, and bacteriological properties of skin exposed to waters of varying pH and ionic strengths over varying time intervals.

25. (U) 72 07 - 73 06 Fingers of 34 volunteers were covered with a surgical glove which was then filled with water. By 48 hours the fingers were wrinkled, swollen and painful to the touch. Swelling began within the first hour of irrmersion and the degree of swelling produced in the first hour varied from one person to another. This condition on the hands is similar to the response of the foot seen in "immersion foot" disease. Sweat duct response on the palms and soles as well as the backs of the fingers and toes is inhibited after the extremity has been immersed in water for an hour. Sweat duct response on the back of the hand and top of the foot, in contrast, is not inhibited after water immersion. Implications of these studies for further investigations of "immersion foot" prophylaxis are discussed.

The Effects of Prolonged Water Exposure on Human Skin

PROBLEM:

Immersion foot results from prolonged immersion of the foot in water. The wrinkled appearance of one's hands and feet after a long bath or after swimming is familiar. However, when feet are continuously immersed in water for two to three days, a portion of people will develop in addition severe pain in their feet. The swollen, white and wrinkled stratum corneum makes walking extremely painful (1). The feet rapidly heal after they are allowed to dry. Studies of this condition have been hampered by the lack of an adequate experimental model and by the necessity to use volunteers for long periods of time, but water seems to be the principal etiologic agent (2).

Some further studies are reported of the effect of water on the skin of the hands and feet, of the uniqueness of the sweat ducts in the skin on the feet and hands, and of an experimental model for immersion foot that can be used to rapidly assay the effectiveness of various agents to prevent immersion foot.

APPROACH:

Volunteer Studies

CPT George Trager, MC

Volunteers: The experiments were performed on 34 healthy male volunteers between the ages of 20 and 53, although most were about 20 years old. Three blacks were included in the sample.

Materials: Plastic film for sweat duct analysis was prepared from Type II silicone-base class 3 "syringe elasticon" manufactured by Kerr Manufacturing Company, Division of Sybron Corporation, Romulus, Michigan. To one ml of the base, 0.1 ml of accelerator was added. The two were mixed then applied to the skin of the volunteers with a wooden applicator stick. After five minutes the material hardened and was removed as an intact, thin film. To count the number of holes in the plastic film, strips of the film were placed in a slide projector and holes in the projected image of the film were counted in a 1 x 2 cm area.

For immersion studies, latex surgeons gloves were sealed to the skin with Dow-Corning Medical Adhesive B.

To induce sweating, the volunteers entered an environmental chamber maintained at 50°C and 95% relative humidity. Most volunteers would sweat within 2 minutes after entering this room.

Skin temperature was measured with a Dermo-Therm infrared detector (Ray-Tek Corporation, Mt. View, Ca.).

RESULTS AND DISCUSSION:

Experimental Immersion Foot: An attempt was first made to produce an immersion foot-like response on the hand. A surgical glove finger was placed over the fourth or fifth finger of six volunteers and made water tight at the metacarpophalangeal joint with adhesive. The glove covering the finger was then filled with 2 ml of tap water so that the finger would be surrounded by water. With a good seal, the finger could remain immersed up to 48 hours.

By 24 hours, the immersed fingers of all 6 volunteers were swollen and wrinkled. At this time most of the water in the glove was gone. Some may have leaked out, but some undoubtedly was absorbed by the fingers. At this time, 2 ml more water were added in the glove.

Two volunteers reported pain in their fingers at 48 hours. Four volunteers had swollen, wrinkled fingers but did not report pain when their fingers were squeezed.

Early Effects of Water on the Hand: Within the first hour, the immersed finger of all volunteers was somewhat swollen and wrinkled. Further, some of the volunteers reacted rapidly to the water with almost immediate wrinkling while others showed a slower response. To quantitate the early response to water immersion, the left hand of 34 volunteers was totally submersed in a pan of water (temperature 47°C) for 1 hour. At intervals during the hour, the circumference of the maximal interphalangeal joint of fingers 2, 3, 4 and 5 was measured with a tape measure. Considerable swelling of this joint occurs within the first hour of immersion; in fact, most of the swelling takes place within 30 minutes of immersion.

The percent swelling of the joint is calculated as

$$\frac{\text{circumference of joint at end of one hour minus circ. of joint at the beginning of the experiment}}{\text{circumference of joint at the beginning of the experiment}} \times 100$$

The average percent swelling can be obtained by averaging the "percent swelling" of fingers 2-5. The average percent swelling was determined for the 34 volunteers. A distribution occurs around the figure of 4% (range 2-11%), with 1 volunteer showing no swelling at all after 1 hour and 2 volunteers who swelled an average of 8%. Table 1 lists the average percent swelling values of 2 volunteers tested 5 different times. While a day to day variation occurs, a clear difference exists between an individual with a marked water effect and one with lesser reaction. Swelling induced by water at 47° was compared to the swelling induced by water at 22°. The degree of swelling depends on the temperature of the immersion water. Thus, all later experiments were performed at the higher temperature.

Sweat Duct Response to Water Immersion: The number of sweat ducts functioning at a given time can be assayed as described by Harris et al (3). A volunteer enters a hot, humid chamber where he remains until he begins to sweat. The area of skin to be tested is then treated with a thin silicone film which hardens within five minutes. However, within minutes of application, small holes appear in the film as the sweat pours through it. Once holes appear, the volunteer leaves the hot environment and the film hardens. It is then peeled off the skin and the number of holes per square cm of the hardened film is counted. Each small hole is produced by the sweat from one sweat duct.

The number of active sweat ducts on selected areas of the hand and foot was counted beofre and after immersion of the extremity in water for one hour. First a basal count was obtained of the sweat response of each volunteer prior to immersion of his extremity in water. Then each volunteer placed one of his hands and one foot in water for an hour. After 1 hour, the wet extremity was removed and carefully dried with a soft towel. An infrared detector was used to assay the temperature of the immersed and dry extremity; and the cooler one was warmed by wrapping it in a towel until

both hands or feet were at the same skin temperature. Then the volunteer entered the sweat chamber and within 2 minutes sweat duct function was assayed.

The average number of sweat ducts functioning was nearly the same on the right or left extremity prior to immersion. Table 2 shows what happened to the sweat duct function on the left hand of one volunteer after immersion in water. It shows that sweating on the palmar surface of the hand and fingers and on the dorsal surface of the fingers is inhibited by water immersion as compared to the function of the ducts on the same areas of the dry hand; but sweating on the dorsal surface of the immersed hand is the same as that on the dorsum of the dry hand. Table 3 shows the data for the same experiment on the feet of the same volunteer. Corresponding to the hand, the sweat ducts of the sole and dorsum of the toes are inhibited while those of the dorsum of the foot are not. Figure 1 shows the areas tested to date on the hands and feet. The shaded areas are those in which sweat duct response is inhibited after water immersion and the clear areas are areas not inhibited. A direct correspondence between the hands and feet exists.

Five volunteers were used in this experiment and all responded to water in the same way. One of the volunteers was a "poor sweller" and one a "good sweller" while the others fell in the middle range. Table 4 contrasts sweat inhibition of the "poor" and "good" sweller. The degree of swelling has no obvious relation to the degree of sweat duct inhibition.

The experiments described are simple to perform and can be extended to a number of areas. For instance, the exact mechanism of the immersion foot response is unknown. However, the response after 48 hours of immersion induced on the fingers of selected volunteers mimics the immersion foot response in that the skin is swollen, wrinkled, and painful to the touch. Further, the swelling portion of this response begins within the first hour of immersion. If one wishes to test various compounds for their ability to prevent immersion foot, one could first test these substances for their ability to prevent swelling of the fingers. In a few hours with enough volunteers one could test large numbers of compounds. The most promising could then be used in the experiments necessary to reproduce true immersion foot. Preliminary experiments in our laboratory indicate that of 15 compounds, MDX 4-4078, a Dow

Corning silicone polymer, is the best compound we have to prevent finger swelling. Independent research indicates this is the best compound available to prevent immersion foot in the field (4).

We could detect no obvious physical differences between men whose fingers swelled and those whose fingers did not. All men were carefully queried as to their history of skin disease but again no correlation is yet obvious between men whose fingers swell and men with particular medical histories.

The relation between the fingers swelling and the sweat duct response is not clear, although both are the result of immersion of the hand or foot in water. Interestingly, in independent experiments, Willis (5) showed that the skin of the dorsal thorax has sweat ducts that are not inhibited by prolonged water immersion. In this respect the skin of the back is like that of the back of the hand. Why do the ducts in the skin of the palm and the sole and back of the fingers behave differently from those elsewhere in the body? The palmar and solar skin is definitely thicker than that on the back of the hand, but this cannot be the explanation for the effects since the skin on the back of the fingers (which also has ducts inhibited by water immersion) is quite thin and at least as thin as that on the back of the hand. Id reactions appear most often on the hands and feet. Many systemic rashes (like rubella) rarely appear on the hands and feet. Thus, this skin is unique on the body and now it has been shown that sweat ducts in those areas are unique in their response to water.

Laboratory Studies

Peter Schmid, Ph.D.

RESULTS AND DISCUSSION:

The finger from a rubber glove was cut off. Medical Adhesive B was sprayed around the finger and the rubber sheet attached firmly. About 0.6 ml of water was injected over the nail into the space between the outer rubber sheet and the finger. The minute puncture of the rubber sheet was sealed with Medical Adhesive B. At 0.5 and 6 hours, the water was removed quantitatively and the volume determined.

Using tritiated water of low specific activity combined with liquid scintillation counting, a method was developed to measure microliter changes in water content due to absorption of water and swelling of the skin surface. The initial average "finger volume" was 608 microliters which decreased to 494 microliters over a period of 6 hours.

In order to monitor possible changes in permeability of the skin, the chloride concentration was also measured. A method using an ion specific electrode was developed which yields quantitative results free of errors due to water soluble amines. Sensitivity is 10^{-5} mM/ml chloride. Using this method it was found that the amount of chloride in the water increased from an average value of 6.810 millimole at 0.5 hours to 9.531 millimole at 6 hours.

It may be concluded that the amount of water decreases by some 19% in 6 hours due to absorption and/or binding due to hydration of the outer layers of the skin.

During the same time interval the amount of chloride increases by 40%. As a result, the chloride concentration increases by some 23%.

FUTURE PLANS:

A full-time investigator will be assigned to this project this summer. Similar experiments will be attempted on volunteers' feet employing waterproof latex socks.

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1. Taplin, D., and Zaias, N.: Tropical immersion foot syndrome. Milit Med. 131: 814-818, 1966.

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3. Harris, D.R., Polk, B.F. and Willis, I.: Evaluating sweat gland activity with imprint techniques. JID 58: 78-84, 1972.

4. Douglas, J.S. and Eby, C.S.: Silicone for immersion foot prophylaxis: Where and hos much to use. Milit Med. 137: 386-387, 1972.

5. Willis, I.: The effects of prolonged water exposure on human skin. JID 60: 166-171, 1973.

Table 1. Day to day variation of "average percent swelling" in two volunteers

Vol.	Average % swelling					
	test 1	test 2	test 3	test 4	test 5	mean value
GWT	10%	7.0%	7.0%	11%	8.0%	8.6%
AK	5.0%	2.5%	3.5%	4.5%	4.5%	4.0%

The circumference of the PIP joints of fingers on the left hand of two volunteers was calculated after immersion of the fingers in warm water for one hour on five separate occasions. An "average % swelling" figure was derived for each test as indicated in the text.

Table 2. The effect of immersion on sweat duct function on the hand

location	No. of functioning sweat ducts per sq cm	
	left hand (immersed)	right hand (dry)
Palm	43,43,34 (avg 40)	38,94,100 (avg 74)
Palmar surface of finger (2nd finger)	32,44,47 (avg 38)	77,50,98 (avg 75)
Dorsum of finger	19,69,25 (avg 37)	64,88,84 (avg 79)
Back of hand	250,260,271 (avg 260)	230,292,276 (avg 266)

A volunteer immersed his left hand in water for one hour. The hand was then dried and the number of active sweat ducts at various sites on the left hand was compared with the number active on the corresponding sites on the right hand.

Table 3. The effect of immersion on sweat duct function on the foot

location	No. of functioning sweat ducts per sq cm	
	left foot (immersed)	right foot (dry)
sole	23,22,25 (avg 23)	114,108,110 (avg 110)
solar surface of toe (2nd toe)	8,1,0 (avg 3)	31,27,10 (avg 22)
dorsum of toe	7,18,0 (avg 9)	21,15,32 (avg 22)
dorsum of foot	165,170,140 (avg 158)	140,161,152 (avg 151)

The same volunteer as in Table 2 kept his left foot immersed in water for one hour. The foot was then dried and the number of active sweat ducts at various sites on the left foot was compared with the number active on the corresponding sites on the right foot.

Table 4. Sweat duct inhibition compared in "good swellers" and "poor swellers"

Vol.	Avg % swelling in one hour	No. of functioning sweat ducts per sq cm	
		immersed hand	dry hand
RS	1.6%	17,15,5 (avg 12)	110,194,150 (avg 151)
CP	5.0%	27,9,15 (avg 17)	116,120,151 (avg 129)
GT	9.0%	24,11,12 (avg 16)	87,59,55 (avg 67)

This table contrasts the sweat duct inhibition found in persons whose fingers rapidly swell when immersed in water with the response in persons whose fingers swell more slowly when immersed.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6790	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A871	00	001
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Delayed Type Skin Reaction and Lymphocyte Transformation in Cutaneous Diseases (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 - Clinical Medicine; 010100 - Immunology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 04	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PREVIOUS 73	1	128.8
FISCAL YEAR CURRENT 74	1	120.0

19. RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Institute of Research
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21. GENERAL USE

20. PERFORMING ORGANIZATION
NAME: Letterman Army Institute of Research
Dermatology Research Division
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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TELEPHONE: 415-561-3006
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) intradermal skin test; (U) lymphocyte transformation; (U) leukocytes; (U) phytohemagglutinin; (U) delayed hypersensitivity; (U) volunteers

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To develop in vitro immunologic correlates to the cutaneous fungal infections in humans.

24. (U) Volunteers will be skin tested with commercially available fungal antigen and divided into sensitized and non-sensitized groups. Circulating lymphocytes obtained by venapuncture from individuals in both groups will be challenged in vitro with specific antigen prepared from cutaneous disease producing organisms. Cultures will be followed for 5 days utilizing standard lymphocyte transformation procedures to determine which antigens are capable of inducing a lymphablastic change.

25. (U) 72 07 - 73 06 We have established that the glycopeptides extracted from T. mentagrophytes by Cruickshank is a locally nonirritating, nontoxic antigen suitable for use in animal and human studies both in-vivo and in-vitro. Positive delayed sensitivity skin test responses to this antigen are seen in about 65% of adult males, and 92% of those showing a positive skin test response have no clinical problems with tinea pedis. In contrast, about 75% of those individuals in whom immediate reactions are seen are chronically infected with dermatophytic fungi. Most of the individuals who have an immediate reaction to trichophytin do not have a delayed response or show a significantly smaller size reaction.

Delayed Type Skin Reaction and Lymphocyte Transformation in Cutaneous Diseases

Henry E. Jones, LTC MC

PROBLEM:

Cutaneous fungal infections are common problems in military medicine. Under adverse environmental conditions like the hot and humid tropics, cutaneous fungal infections may become disabling to the individual, even rendering a division ineffectual. Presently no effective prophylactic or therapeutic agents are available that prevent cutaneous fungal infections from reaching epidemic proportions. The interaction between the infectious agent and the host is poorly understood, but it appears that the immunologic reactions that these organisms initiate in man are significant in the host-parasite relationship.

OBJECTIVE:

The purpose of this work unit is to study the immunologic relationships between these infectious agents and man. The primary goal of the studies have been to develop in vitro immunologic correlates that will detect some of the same parameters that are detected by skin testing with fungal antigens. Preliminary to actually studying in vitro immunologic correlates to the cutaneous fungal infections in humans, a similar study was undertaken using tuberculin. The results of this pilot study (Amer Rev Resp Dis 107: 530, 1973) indicated that the level of skin sensitivity and degree of lymphocyte transformation in vitro correlated quite closely in most cases. This indicates that the in vitro immunologic correlates can be used to monitor the state of allergic sensitivity, thus avoiding skin testing.

A similar study of in vivo and in vitro fungal antigens will be conducted. However, progress in this area has been hampered due to shortage of the appropriate fungal antigen for use in the in vivo and in vitro studies.

RESULTS:

In the past 1.5 years, we have established that the glycopeptide extracted from T. mentagrophytes by

Cruickshank is a locally nonirritating, nontoxic antigen suitable for use in animal and human studies both in vivo and in vitro. Positive delayed sensitivity skin test responses to this antigen are seen in about 65% of adult males, and 92% of those showing a positive skin test response have no clinical problems with tinea pedis. In contrast, about 75% of those individuals in whom immediate reactions are seen are chronically infected with dermatophytic fungi. Most of the individuals who have an immediate reaction to trichophytin do not have a delayed response or show a significantly smaller size reaction.

Individuals who are susceptible to chronic or recurrent dermatophytic infection have been studied in the Letterman Army Medical Center Dermatology Clinic using trichophytin plus several other antigens. Studies in these clinic patients have shown the same pattern of allergic sensitivity detected in the prison inmate studies mentioned above. Furthermore, the studies conducted at Letterman used several other delayed type hypersensitivity antigens (Candida, PPD, SKSD, mumps and poison ivy); and it was found that the patients with chronic fungal infections and hypo-responses to trichophytin reacted normally to the other antigens. This indicates that there is a specific defect in patients with chronic fungal infections such that they do not respond with cell mediated immune responses to the infecting fungi.

A skin test, clinical and mycological survey of a group of atopic individuals in the Center for Asthma Research Institute and Hospital (CARIH), Denver, Colorado, indicated that although none of the subjects were infected in the past or at present with dermatophytic fungi, 40% showed immediate wheal and flare skin test reactions to the trichophytin. Most of the individuals who showed immediate reactions to the trichophytin antigen also showed reactions to noninfectious airborne molds and fungi, indicating that the immediate reactions to trichophytin are probably an immunologic cross-reaction. We take this as evidence that the atopic individual is predisposed to develop immediate reacting antibody to antigens produced by the pathogenic and nonpathogenic fungi. The failure of these same individuals to develop delayed sensitivity may relate to their susceptibility in later life to cutaneous fungal infections.

Operating under the hypothesis that the reason for the chronic fungal infections is a deficiency of cell-mediated immune responses to the fungi, an attempt was made using transfer factor to induce delayed sensitivity to trichophytin in nonreactive subjects. The pilot study using transfer factor prepared from an individual who had been experimentally infected with dermatophytic fungi (see Annual Report 006, Experimental Fungus Infections in the Skin of Man) was successful in one of two individuals, and the delayed sensitivity to trichophytin has persisted for six months since the transfer. In a subsequent larger study designed to passively transfer delayed sensitivity with transfer factor and to correlate this with susceptibility to experimental infection, there was a failure of the transfer factor to work. The reason for this failure is under investigation.

Lymphocyte transformation using fungal antigens have been accomplished in this laboratory several times during the last two years utilizing Cruickshank's trichophytin and several other antigenic extracts from the dermatophytic fungi. Specific transformation has been obtained using Cruickshank's trichophytin, PEGB, CM104, CM105, CM106, and crude ethylene glycol extracts of fungal mycelia. Extensive studies have not been possible due to small quantity of these antigens available. Recently (see Annual Report - Biochemical Mechanisms of Fungal Pathogenesis) larger quantities of an ethylene glycol extractable antigen have become available. This antigen we have numbered CM107, and recent evidence indicates that the antigen is slightly more potent than Cruickshank's trichophytin for skin testing and that it works well in in vitro lymphocyte studies. The antigen is available in quantity.

With the addition of a new investigator, lymphocyto-toxin studies are being initiated to determine if antigen stimulated lymphocytes produce factors which may exert a toxic effect upon mammalian cells and more specifically upon microorganisms such as the pathogenic dermatophytic fungi. Failure of sensitized host lymphocytes to produce lymphocytotoxin properly during infection could account for the inability of the host to rid himself of the infection.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6783	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. GEOG'N INSTN'N	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3A062770A841	00	004
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Biochemical Mechanisms of Pathogenesis in Fungal Skin Infections (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 - Biochemistry; 010100 - Microbiology; 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 11	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 73	5	54.8
CURRENT FISCAL YEAR 74		60.0

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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TELEPHONE: 415-561-2921

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ASSOCIATE INVESTIGATORS

NAME: Jaeger, R.R., DAC

NAME: Ottaviano, P.J. SP5 DA

21. GENERAL USE

22. KEYWORDS (Precede Each with Security Classification Code) (U) skin
(U) stratum corneum; (U) enzymes; (U) antigens; (U) immunogens; (U) virulence

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) 1) Elucidate biochemical mechanisms of infection. 2) Perfect assay of enzymes capable of hydrolyzing substrates found in stratum corneum; characterize isolated enzymes by physical-chemical procedures. 3) Correlate virulence, enzyme production, and nutritional requirements of fungus with morphological and physiological variations in vitro and in vivo. 4) Determine antigenic activity of isolated enzymes. 5) Isolate and purify fungal antigens and fungal toxins. 6) Devise novel therapeutic and preventive measures by perfecting antigen isolation procedures for use in immunization studies.

24. (U) 1) Nutrition - compare growth and metabolism of fungus in vitro utilizing compounds present in stratum corneum; determine nutritional requirements of various dermatophytes. 2) Antigens (Vaccine) - isolate and purify cellular fractions capable of conferring immunity to experimental and natural dermatophyte infections in animals and man. 3) Produce cell wall-defective variants of dermatophytes to study immunogenicity.

25. (U) 72 07 - 73 06 1) The amino acid, vitamin, salt, and carbohydrate requirements of T. mentagrophytes have been determined. 2) A defined growth medium for the culture of dermatophytes has been developed. 3) Specific dermatophyte antigen for delayed-type skin reactions has been purified and characterized chemically and immunologically. 4) Cell membrane and cytoplasmic antigens have been isolated and are being characterized.

* Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

Biochemical Mechanisms of Pathogenesis in Fungal Skin Infections

David J. Bibel, CPT, MSC
June R. Jaeger, DAC
Paul J. Ottaviano, SP5

PROBLEM:

The clinical significance of dermatophytic fungal infections of combat personnel in tropical climates has been well documented (1-4). Trichophyton mentagrophytes (Arthroderma benhamiae) has been shown to be the primary etiologic agent for most of the debilitating fungal infections in U.S. Army personnel in RVN (1).

There is, however, a paucity of definitive information concerning the specific mechanisms of pathogenesis which enable the dermatophytic fungi to cause disease in the skin.

APPROACH:

The purpose of this study is to elucidate the biochemical mechanisms that T. mentagrophytes utilizes when infecting human stratum corneum. Knowledge of the specific mechanisms which allow the fungus to invade and colonize the human skin will permit the evaluation of new anti-fungal agents designed to alter or inhibit the fungal pathogenic mechanisms.

Areas currently under investigation are: the nutritional requirements of Trichophyton mentagrophytes and other dermatophytes; the isolation of specific fungal enzymes capable of attacking macromolecular constituents of the stratum corneum; the isolation and purification of antigenic fractions from T. mentagrophytes; and the isolation and identification of toxins and/or primary irritant factors produced by dermatophytes.

RESULTS AND DISCUSSION:

Determining the specific carbohydrate, vitamin, salt, and amino acid requirements of T. mentagrophytes has led to the development of a defined growth medium for the culture of dermatophytes in vitro. This medium

yields approximately equivalent dry weights of fungus when compared to well-known complex media, and has been better characterized than previous defined media developed in this laboratory. Attaining this medium is a critical step in the design of future experiments into the antigenicity, toxicity, and biochemistry of dermatophytes.

Antigen extracts of Trichophyton mentagrophytes have been purified and characterized chemically, electrophoretically, and immunologically both in vitro and in vivo. The glycopeptide antigen has been shown to be potent and dermatophyte specific by cutaneous delayed-type hypersensitivity responses and in vitro lymphocyte transformation tests. The role of this antigenic material in the fungus is under investigation as well as its ability to confer immunity to experimental fungal infections. Preliminary attempts of inducing protoplastic forms of the fungus were made with limited success. If the antigen is part of the wall structure, then protoplasts of dermatophytes should be nonimmunogenic.

FUTURE PLANS:

Utilize the defined medium to isolate and characterize fungal enzymes and toxins and to conduct comparative, quantitative investigations of dermatophyte antigens. Develop chemical and immunological methods for standardization of fungal antigens. Determine location and role of antigenic glycopeptide in the fungus.

REFERENCES:

1. Blank H, Taplin D, Zaias N: Cutaneous Trichophyton mentagrophytes infections in Vietnam. Arch Derm 99: 135-144, 1969.

2. Blank H, Zaias N, Taplin D: Final Report: Dermatological Team of the Armed Forces Epidemiological Board, 1968.

3. Sanderson PH, Sloper JC: Skin Diseases in the British Army in S.E. Asia. II: Tinea corporis. Brit J Dermatol 65: 300-309, 1953.

4. Sanderson PH, Sloper JC: Skin Disease in the British Army in S.E. Asia. III: The relationship between mycotic infections of the body and of the feet. Brit J Dermatol 63: 239-251, 1953.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6794	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91	00	006
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Experimental Fungus Infections in the Skin of Man: A Therapeutic Model (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 - Microbiology; 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 11	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 73	2	5.8
FISCAL YEAR CURRENT 74	2	10

19. RESPONSIBLE DOD ORGANIZATION

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NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) dermatophytosis; (U) experimental infection; (U) skin; (U)human volunteers.

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) 1) To perfect a reproducible, experimental fungal infection of the skin of humans. 2) To determine the minimum number of fungal spores required to produce persistent infections in humans with and without pre-existing immunity to dermatophytic fungi. 3) To correlate the degree of immunity in human volunteers with the severity of the induced infections. 4) To prepare a protective vaccine that will prevent or minimize the severity of experimental and natural infections. 5) To evaluate prophylactic and therapeutic anti-fungal agents.

24. (U) To develop an experimental model infection that is quantifiable and reproducible from site to site in any one individual and from subject to subject.

25. (U) 72 07 - 73 06 We have established that most virginal human subjects acquire a specific immunity from their first infection which will increase their resistance to a second experimental infection. This specific acquired immunity correlates closely with delayed sensitivity responses to antigens extractable from these fungi. It appears from these same studies that 80% of those subjects experiencing natural dermatophytic infections develop a similar protective immunity and are not bothered by further clinical problems with dermatophytic fungi. In contrast, those individuals who remain chronically infected following their natural dermatophytic infection show decreased cell mediated responses to the antigens extractable from the dermatophytic fungi, and more frequently show immediate wheal and flare skin test responses which are mediated by circulating antibody, probably of the IgE class.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

Experimental Fungus Infections in the Skin of Man: A Therapeutic Model

Henry E. Jones, LTC MC
David J. Bibel, CPT MSC

PROBLEM:

Cutaneous fungal infections are common problems in both civilian and military medicine. In the military, under adverse environmental conditions such as in the hot and humid tropics, cutaneous fungal infections may become disabling to the individual, even rendering a fighting force ineffectual. Presently no practical and effective prophylactic or therapeutic agents are available that prevent cutaneous fungal infections from reaching these epidemic proportions. The interaction between the infectious agent and the host is poorly understood, but it appears that the immunologic reactions that these organisms initiate in man are a significant part of the host-parasite relationship.

APPROACH:

The approach followed under this work unit has been to develop an experimental model infection that is quantifiable and reproducible from site to site in any one individual and from subject to subject. This has permitted us to study the natural course of experimental infections in animals and humans. These studies have been correlated closely with other work units included in this annual report which permits studying the course of experimental infections in the three major immunobiological subclasses of susceptibility to cutaneous fungal infection. This model also lends itself to the study of prophylactic and therapeutic agents in animals and humans, and similarly permits study of the biochemical and ecological skin factors which may affect susceptibility to infection.

RESULTS:

During the past 1.5 years, this laboratory has established that most virginal human subjects acquire a specific immunity from their first infection which will increase their resistance to a second experimental infection. This specific acquired immunity

correlates closely with delayed sensitivity responses to antigens extractable from these fungi. It appears from these same studies that 80% of those subjects experiencing natural dermatophytic infections develop a similar protective immunity and are not bothered by further clinical problems with dermatophytic fungi. In contrast, those individuals who remain chronically infected following their natural dermatophytic infection show decreased cell mediated immune responses to the antigens extractable from the dermatophytic fungi, and more frequently show immediate wheal and flare skin test responses which are mediated by circulating antibody, probably of the IgE class.

During this fiscal year we have continued to follow one virginal atopic individual who was experimentally infected on his forearm and whose infection healed very slowly. This subject subsequently developed an infection on his feet from which the same organism could be recovered. This individual maintained an infection for some 10 months despite intermittent treatment with griseofulvin. This individual is atopic and developed an immediate wheal and flare response as well as a delayed response during the course of his infection, confirming the association between susceptibility to dermatophytosis, atopy and immediate wheal and flare skin test sensitivity.

A transfer factor study was conducted to determine if a state of delayed sensitivity and resistance to experimental dermatophytic infection could be passively transmitted to 15 virginal individuals; but, as mentioned in Annual Report to work unit 001, this transfer factor did not confer a state of delayed sensitivity on any subject, including the controls. At present it is not understood why the transfer factor did not work, since a 2-subject pilot study preceding the large study had been conducted with positive results in 1 subject. It is suggested that possibly virginal subjects are not susceptible to transfer factor, but only those individuals who have had some experience with the organism and have a quiescent sensitivity are actually boosted by transfer factor therapy. This hypothesis is presently being tested.

A series of prophylactic and therapeutic studies utilizing the experimental infection model were conducted in cooperation with Dr. William Epstein and

Dr. Sidney Riegelman at the University of California San Francisco Medical Center. The first of these block designed placebo controlled studies involved the comparison of oral and topical griseofulvin as a prophylactic agent. The griseofulvin was administered orally four times a day, and topically in an ethanol solution to the sites to be infected four consecutive days prior to inoculation of the infectious agent. The results of this study clearly indicate that topical griseofulvin was a very effective prophylactic agent, since none of the individuals receiving this therapy became infected. Surprisingly, oral griseofulvin was not an effective prophylactic in this study. Subsequently, in a similar block design placebo controlled study, topical griseofulvin in alcohol was tried as a therapeutic agent with experimental infections initiated in the three immunobiological subclasses defined in this year's Annual Report 001. All of the data have not been analyzed, but it appears clear that griseofulvin as a therapeutic agent administered daily from the third to the tenth day during the course of an experimental fungal infection provides little, if any, therapeutic effect. The conclusions from these two studies should be that griseofulvin could possibly be used topically as an effective prophylactic agent for dermatophytosis.

To further investigate the hypothesis that certain individuals are predisposed to develop dermatophytic infections, we have studied the HL-A haplo type of subjects in the three immunobiologic classes. To date, only ten members of each of the three immunobiologic classes have been studied, but it does not appear that there is any correlation between HL-A haplo type and susceptibility or resistance to natural and experimental infections. Other studies involving these three immunobiological subclasses are also underway and we can state that there may be increased quantities of extractable skin lipids on the feet of virginal subjects. There is some indication from crude anthropometrical studies of the feet of the three immunobiological classes to suggest that those individuals most resistant to natural infections have longer toes and wider interweb spaces, i.e., less moisture and maceration.

MAXILLOFACIAL SCIENCES DIVISION

Program Element 6.11.02.A
Defense Research Sciences, Army

Project Number 3A061102B71R
Dentistry

Task Area Number 04

- - - - - - - - - - - - - - - - -

Program Element 6.21.10.A
Bio-Medical Investigations

Project Number 3A062110A825
Oral & Maxillofacial Sciences

Task Area Number 00

MAXILLOFACIAL SCIENCES DIVISION

This division's research program is directed toward developing methods for reducing the morbidity associated with traumatic injuries to the maxillofacial area and oral diseases prevalent in soldiers.

Lack of personnel and economic resources have required termination of three of the research studies and limited activities in other areas. Despite these limitations, major advances have been made in the following research studies: Prevention of Post-Extraction Alveolitis; Bone Repair; Mandibular Bone grafts; Oral and Maxillofacial Wound Infection; Actinic Blocking Agents for Protection of Lip Mucosa; Survey of Oral and Maxillofacial Injuries in the Federal Dental Services.

Rinsing of the oral cavity and the area around the lower third molar tooth with an antiseptic mouthwash prior to its extraction has been shown to significantly reduce the incidence of complicating post-extraction alveolar osteitis. This simple method is applicable to dental practice in the field as well as fixed installations and is being promulgated for use throughout the Army.

An in vivo laboratory animal model for studying physical strength of bone repair sites has been developed and evaluated. This method is quantitative, reproducible, and sufficiently sensitive to allow comparative evaluation of clinical procedures with statistically confident results. Additional studies are in progress to correlate physical strength with other clinical and laboratory parameters.

Improved methods for processing and sterilizing lyophilized allogeneic decalcified bone have been developed for mandibular bone grafts.

Studies on the bacterial spectrum of oral and maxillofacial wound infection indicate that a significant number of gram positive bacterial strains resistant to penicillin are being cultured from such lesions in patients not receiving antibiotics. These findings emphasize the need for epidemiologic data as a clinical guideline in therapy of such infections where an empirical approach is necessary.

The most effective commercially available actinic blocking agents for protection of the lip mucosa have been identified and their limitations noted. Procedures are being initiated to standardize applicable agents for military procurement and use.

The incidence and types of maxillofacial injuries are being studied in the five Federal Services. Data on over six thousand maxillofacial injuries have been received. Computer programing is being accomplished to allow in-depth analysis of these injuries and on line computation.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6780	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	04	135
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with security classification code)

(U) Oral Disease in Military Populations (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

Medicine

002600 - Biology; 012900 - Physiology; 010100 - Microbiology; 003500-Clinical

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 07	74 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 73	9	63
CURRENT FISCAL YEAR 74	9	40

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

NAME: Akers, W.A., COL MC

TELEPHONE: 415-561-5181

21. GENERAL USE

20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Maxillofacial Sciences Division
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Lilly, G.E., COL DC

TELEPHONE: 415-551-5160

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Cutcher, J.L., LTC DC

NAME: Tempel, T.R., MAJ DC DA

22. KEYWORDS (Precede each with security classification code) (U) effect of electric stimulation on bone repair; (U) endodontics; (U) alveolitis; (U) bacteremia; (U) bone repair; (U)military patient

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security classification code.)

23. (U) To reduce morbidity and non-effectiveness of affected Army personnel by establishing methods, practical under military conditions, for more efficient treatment, diagnosis and prevention of selected oral diseases prevalent in the military.

24. (U) Human clinical investigations and laboratory studies.

25. (U) 72 07 - 73 06 Investigations are being conducted in 4 sub-projects: Endodontics in Military Dentistry; Prevention of Post-Extraction Alveolitis; Bone Repair; Effect of Electric Stimulation on Bone Repair.

See specific DD Form 1498 for current status of each subject.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6805	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	H. Terminate	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	04	143
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)*

(U) Endodontics in Military Dentistry (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 01	73 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	0	2.0
	CURRENT 74	0	0

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Letterman Army Institute of Research

ADDRESS:* Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

NAME: Akers, W.A., COL MC

TELEPHONE: 415-561-5181

21. GENERAL USE

20. PERFORMING ORGANIZATION

NAME:* Letterman Army Institute of Research
Maxillofacial Sciences Division

ADDRESS:* Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME:* Robert, R., CPT MC

TELEPHONE: 415-561-4042

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Lilly, G.E., COL DC

NAME: Freeze, K., SP4 DA

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) endodontics; (U) root canal; (U) dentistry; (U) military oral health; (U) human teeth; (U) canine teeth;

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) To improve the oral health of the Army. This study is directed toward simplifying and reducing the time necessary to accomplish root canal therapy in the teeth of soldiers, thereby reducing the number of tooth extractions required.

24. (U) Laboratory studies on animals and extracted human teeth.

25. (U) 72 07 - 73 06 Due to budgetary and personnel limitations and higher priority of other research, no investigations have been conducted in this area. This project is being terminated.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6795	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71F	04	144
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Prevention of Post-Extraction Alveolitis (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 01	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PREVIOUS 73	2	1.0
FISCAL YEAR CURRENT 74	2	10

19. RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Institute of Research
ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL
NAME: Akers, W.A., COL MC
TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION
NAME: Letterman Army Institute of Research
Maxillofacial Sciences Division
ADDRESS: Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Lilly, G.E., COL DC
TELEPHONE: 415-561-5160
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Osbon, D.B., COL DC
NAME: Rael, E.M., DAC DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) alveolar osteitis; (U) mandibular third molar; (U) extraction; (U) antiseptic/ mouthwash; (U) military dentistry

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Although the incidence has not been established in the US Army, available data suggest that at least 2 percent of all tooth extractions are followed by local infection of the tooth socket (alveolitis or "dry socket"). Ninety percent of the cases of alveolitis that do occur follow the removal of a mandibular third molar (wisdom tooth). The objective of this study is to develop a simple, effective method of preventing this complication, that is practical in military situations.

24. (U) Prevention of post-extraction alveolitis by use of a topical antiseptic to temporarily reduce the bacterial population of the oral cavity. A collaborative study between Letterman Army Medical Center, Letterman Army Institute of Research and Oakland Naval Hospital.

25. (U) 72 07 - 73 06 The incidence of alveolar osteitis in 2,195 third molar extractions was 9.1 percent. Factors predisposing to alveolar osteitis were oral contraceptives for women and surgical extractions. Pericoronitis, intra venous sedation and the use of pre-operative antibiotics did not significantly affect the incidence of alveolitis. The use of an oral lavage with a topical antiseptic did not significantly reduce the incidence of alveolar osteitis.

Prevention of Post-Extraction Alveolitis

Colonel Gilbert E. Lilly, DC
Colonel Donald B. Osbon, DC
Miss Erlinda Rael DAC

PROBLEM:

The incidence of alveolitis (dry socket) is not established in the U.S. Army. Available data suggest that approximately 2 percent of all tooth extractions, and 10-25 percent of all mandibular third molar (wisdom tooth) extractions, are followed by this complication. Furthermore, 90 percent of the dry sockets that do occur are in the mandibular third molar region. Based on the current Army population and dental treatment required, 40,000 cases of alveolar osteitis are estimated to occur annually. This results in an annual loss of 160,000 patient man-hours and creates a dental workload of 80,000 separate additional appointments.

While the precise etiology of alveolitis has not been established, 3 contributory factors are recognized: (1) the general systemic status of the patient (age, nutrition, systemic disease, and medications); (2) local trauma; and (3) local microorganisms. The systemic status of the patient and the amount of local trauma are factors which are unique to each patient and each extraction. Because of this, their inherent variability makes them extremely difficult to control or standardize in studying a series of patients. Although the specific types and numbers of microorganisms vary from one case to another, their presence is constant. Topical oral antiseptics are known to drastically reduce the oral bacterial population for periods of up to 1 1/2 hours.

This study tests the hypothesis that a topical antiseptic can effect a reduction in the oral bacterial population during the time interval in which surgery and subsequent clot formation occur, and that such a reduction is a critical factor in preventing alveolar osteitis.

APPROACH:

Patients requiring mandibular third molar extractions are randomly placed in one of 2 groups: Experimental and Control. Patients in the experimental group have their mouths and gingival sulci in the mandibular second and third molar areas lavaged with an antiseptic mouthwash prior to third molar extraction. Patients in the control group receive no such pre-extraction lavage. In all other respects, patients in the control group and experimental group receive comparable treatment before, during and after surgery. Basic information concerning the patient, the extraction and group is recorded on a special card. Postoperatively, all patients are evaluated for alveolar osteitis. The investigator conducting the examination is not aware of the patient's group allocation (experimental or control), and findings are recorded on a separate data card. For the purpose of the investigation, alveolar osteitis is defined as any case with localized post-extraction pain which requires treatment with a local obtundent dressing. Collected data is processed with electronic data processing equipment as the basis for determination of the incidence of alveolar osteitis. Statistical validity is determined by Chi-Square analysis. This collaborative study involves Letterman Army Institute of Research, Letterman General Hospital, and Oakland Naval Hospital.

RESULTS:

A total of 1358 patients were included in this study. They ranged in age from 15 to 75 years. Over 85 percent, however, were in the second and third decades of life and 69 percent were males. Birth control pills were used by 28.2 percent of the women and 5.6 percent of the patients received antibiotics preoperatively. In almost all cases, antibiotics were prescribed for prophylactic coverage because of systemic disease. Local anesthesia was used in all cases and adjunctive intravenous sedation was used in 58 percent of the cases.

A total of 2,195 mandibular third molars were extracted. Three-hundred fifty-nine of these extractions were simple (non-surgical extractions) and 1836 (83.6 percent) were surgical extractions. The extractions were performed

by 30 different individuals. Six were board certified oral surgeons, eight were oral surgery residents and 16 were rotating dental interns. Acute pericoronitis was associated with 3.8 percent of the teeth and chronic pericoronitis with 40.1 percent of the teeth at the time of extraction.

Alveolar osteitis occurred in 200 of the 2195 mandibular third molar extraction sockets for an overall incidence of 9.1 percent.

Identified factors which predisposed patients to alveolar osteitis were women taking oral contraceptives and surgical extractions as compared to simple extractions. The incidence of alveolar osteitis in women taking oral contraceptives was nearly 3 times greater than in women not taking oral contraceptives (21.4 percent as compared to 7.2 percent). This finding was significant with a P value of less than 0.025 (Table 1). This was also true for all observed internal varibles except simple extractions and acute pericoronitis.

The incidence of alveolitis for simple extractions was 3.9 percent, and 10.1 percent for surgical extractions (Table 2). This observation was significant with a P value of less than 0.025. The internal variables: I.V. sedation, preoperative antibiotics, oral lavage and acute pericoronitis had no statistically significant effect on the incidence of alveolitis. With the exception of acute pericoronitis, however, the incidence of alveolar osteitis was less for all observations in simple extraction cases.

Possible predisposing factors not judged to be statistically significant were pericoronitis, preoperative antibiotics, and I.V. sedation.

The incidence of alveolar osteitis was 7.2 percent for acute pericoronitis, 7.9 percent for chronic pericoronitis and 10.2 percent in cases without pericoronitis (Table 3). Comparison of acute and chronic pericoronitis cases to cases not affected by pericoronitis was not statistically significant. Significant internal variables for chronic pericoronitis cases as compared to cases without pericoronitis were found with men, women taking oral contraceptives,

patients given I.V. sedation, and patients not having oral lavage.

The use of an oral lavage, as described in this study, significantly reduced the overall incidence of alveolar osteitis (Table 4). The P value was less than 0.025 with an observed incidence of alveolitis of 7.4 percent in the lavage group and 10.5 percent in the no lavage cases. With the exception of simple extractions, the incidence of alveolitis was reduced for all internal variables in cases receiving oral lavage. Statistically significant observations were for surgical extractions, no pericoronitis, IV sedation, and no preoperative antibiotics.

DISCUSSION:

These data indicate that women taking oral contraceptives have a marked predilection for alveolar osteitis and that surgical extractions appear to be associated with a higher incidence of alveolitis. The presence of pericoronitis does not appear to increase the incidence of alveolitis and pericoronitis should not therefore preclude tooth extraction.

Preoperative antibiotics did not prevent alveolitis in this study nor did they appear to markedly reduce its incidence (Table 5).

The use of an oral lavage with an antiseptic appears to be of value in reducing the incidence of alveolar osteitis. It is a simple technique which can be used in combination with other preventive agents which might prove of further value. In addition, it has been previously demonstrated that such lavage reduces the incidence of post-extraction bacteremia.

SUMMARY:

The incidence of alveolar osteitis in 2,195 third molar extractions was 9.1 percent. Factors predisposing to alveolar osteitis were oral contraceptives for women, and surgical extractions. Pericoronitis, I.V. sedation and the use of preoperative antibiotics did not significantly affect the incidence of alveolitis. The use of an oral lavage with a topical antiseptic did significantly reduce the incidence of alveolar osteitis.

FUTURE PLANS:

This study has been modified and is being continued. Experimental cases receiving oral lavage are being given a topical antiseptic mouthwash with instructions to rinse the mouth four times a day for two days after surgery.

TABLE 1

WOMEN & ORAL CONTRACEPTIVES

NO CONTRACEPTIVE			ORAL CONTRACEPTIVE		
Alveolitis			Alveolitis		
		Extraction			
10.6	47	Simple	14	21.4	
6.8	442	Surgical	178	21.3	P<0.025
		Pericoronitis			
18.2	11	Acute	6	33.3	
9.6	167	Chronic	43	37.2	P<0.025
5.5	311	None	143	16.1	P<0.025
		Lavage			
6.1	246	Yes	107	16.8	P<0.025
8.2	243	No	85	27.1	P<0.025
7.2	489	Total	192	21.4	P<0.025

TABLE 2

EXTRACTION

SIMPLE			SURGICAL		
Alveolitis			Alveolitis		
		Sedation			
6.3	112	Yes	1160	9.7	
2.8	247	No	676	10.9	P<0.025
		Antibiotic			
0	17	Yes	106	7.5	
4.1	342	No	1730	10.3	P<0.025
		Lavage			
5.8	137	Yes	872	7.7	
2.7	222	No	964	12.3	P<0.025
		Pericoronitis			
10.0	10	Acute	73	6.8	
4.0	173	Chronic	708	8.9	P<0.05
3.4	176	None	1055	11.2	P<0.025
3.9	359	Total	1836	10.1	P<0.025

TABLE 3

PERICORONITIS

	NUMBER OF EXTRACTIONS	CASES OF ALVEOLITIS	PERCENT AFFECTED BY ALVEOLITIS	
Acute	83	6	7.2	P>0.05
Chronic	881	70	7.9	P>0.05
None	1231	125	10.2	

TABLE 4

LAVAGE

WITH Alveolitis			WITHOUT Alveolitis		
		Extraction			
6.7	119	Simple	239	2.5	
7.5	889	Surgical	947	12.6	P<0.025
		Pericoronitis			
3.6	56	Acute	27	11.1	
7.0	417	Chronic	464	8.8	
8.2	536	None	695	11.7	P<0.05
		Sedation			
7.6	593	Yes	679	10.9	P<0.05
7.2	416	No	507	10.1	
		Antibiotic			
3.9	51	Yes	72	8.3	
7.6	958	No	1114	10.7	P<0.025
7.4	1009	Total	1186	10.5	P<0.025

TABLE 5

PRE-OP ANTIBIOTIC

YES			NO	
Alveolitis			Alveolitis	
		Extraction		
0	17	Simple	342	4.1
7.5	106	Surgical	1730	10.3
		Pericoronitis		
0	12	Acute	71	8.5
8.3	48	Chronic	833	7.9
6.3	63	None	1168	10.4
		Lavage		
3.9	51	Yes	958	7.6
8.3	72	No	1114	10.7
6.5	123	Total	2072	8.8

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6910	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	H. Terminate	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	04	142
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Effect of Electric Stimulation on Bone Repair (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 03	73 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	2	1.0
	CURRENT 74	0	0

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

NAME: Akers, W.A., COL MC

TELEPHONE: 415-561-5181

21. GENERAL USE

20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Maxillofacial Sciences Division
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Lilly, G.E., COL DC

TELEPHONE: 415-561-5160

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Robert, R.C., CPT DC

NAME: Freeze, K., SP4 DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) mandibular fractures; (U) bone repair; (U) piezoelectricity; (U) wound healing

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) 1) Reduce the hospitalization time and military manhours lost due to fractures of maxillofacial bones. 2) Determine if bone repair can be accelerated by small amounts of electric current.

24. (U) Reduction of the number of oral bacteria in humans by use of an antiseptic mouthwash solution prior to specific gum treatments.

25. (U) 72 07 - 73 06 In vivo studies on dogs have been accomplished. A functional animal laboratory model has been developed and a dependable, adjustable constant flow electric circuit has been perfected and applied. This project is being terminated because of transfer of the principal investigator.

*Available to contractors upon originator's approval.

Effect of Electric Stimulation on Bone Repair

Colonel Gilbert E. Lilly, DC
Captain Richard C. Robert, DC
SP4 Kenneth Freeze

PROBLEM:

Accepted treatment for fractures and bone grafts of the jaws usually involves wiring the jaws together. Consequently, it is necessary to hospitalize the patient until bone repair has progressed to the point that the wires can be removed and the patient can eat a normal diet and is not in danger of aspirating foreign material into the lungs. Such care requires prolonged hospitalization and results in loss of many manhours.

A number of authors have reported and measured electric potentials which are apparently due to a piezoelectric effect in human and other mammalian bones when bones are subjected to mechanical stress in in vitro laboratory studies. More recently, in vivo laboratory studies and selected applications on humans have resulted in reports of increased and more rapid bone deposition in bones subjected to small-amperage, direct electric current. The exact amount of current necessary and the relation of bone formation to the implanted electrodes are not well established.

This study is being undertaken to: (a) determine the effect of electric current on bone repair; (b) determine the most appropriate amount and method of delivering electric stimulation to the mandible to accelerate bone repair; (c) reduce the hospitalization time and military manhours lost due to mandibular fractures.

APPROACH:

Three bilateral mandibular surgical defects were prepared by way of an aseptic extra-oral surgical approach on 8 dogs. Each defect was 1 mm wide and 2 cm apart, cut through the inferior border of the mandible. Each defect was extended to, but not through, the mandibular canal. Direct electric current across the

center defect was furnished initially by a circuit similar in design to that of Lavine et al., using a 1.4 volt E400 Eveready Gh battery connected in series with a 170 K OHM 10 resistor (resistance measured prior to use). This power unit was sealed in Silastic Silicone A (Dow Corning Silicone, type A). The copper wire battery leads were fastened to the voltage source with conducting silver epoxy cement (Emerson & Cuming, Gardena, Calif.) One lead had a surface exposure for current monitoring after implant. Platinum electrodes (0.020 inch diameter) were soldered to the copper wire and insulated with shrink tubing. The electrodes were implanted in drill holes (0.5 cm diameter) on either side of the center cut of each defect series. A pair of electrodes were implanted on each side of the mandible, but only those of one side were connected to a power source. Osteogenic activity at the defects was evaluated radiographically and histologically. Dogs were sacrificed 2 and 4 weeks after surgery.

RESULTS:

Numerous technical problems have been encountered in this study, related to the electric circuitry and the animal model. The copper wire leads separated in 3 cases due apparently to metal fatigue, the result of the dogs' constant movement of the neck and jaws. In 2 other cases, circuit failure resulted from interruption of the platinum electrode-copper wire union. Inflammation and infection at the cutaneous entry site of the circuit has been a continual problem necessitating early sacrifice of some animals.

In view of the problems encountered, primary effort has been directed toward developing more reliable and sophisticated electric circuitry and solving problems encountered in the animal model. In cooperation with Rosemount Engineering Corporation, Minneapolis, Minnesota, we have developed an electric power unit and circuitry which is dependable and capable of wider ranges of electric output. In order to avoid the problems encountered with electrode and wire lead failures, this system uses multi-stranded copper leads and cast gold electrodes embedded in RTV 382 silicone.

Using this system with repeated in vitro testing and pre-and postoperative monitoring of in vivo circuits, it has been possible to maintain a constant direct current output of 10 microamperes ± 0.5 microamperes for periods of up to 6 weeks without external leads for monitoring the system.

The constant current source consists of a 16 cell mercury battery providing (when new) 22.4 volts, an F.E.T. transistor and various resistors that a) determine the value of the current, and b) provide a means for measuring it.

The limited current control provided by a single F.E.T. device produces output currents accurate to within +5 percent (0.5 microampere) over the useful range of battery voltages and with load variations ranging from 0 to 1 megohm.

The mercury cell is rated for 45 milliampere hours. This amounts to 4,500 hours of usable life at the 10 microampere discharge rate. The device may be reused until external terminal voltage drops to 16 volts.

DISCUSSION:

Due to the numerous surgical and technical problems encountered, no meaningful experimental observations of the basic effect of the electric circuit on bone repair have been made. It is, however, our belief that the animal and electrical model have been perfected to a point that allows meaningful experimentation directed toward the basic question.

FUTURE PLANS:

This study is being terminated because of transfer of the principle investigator. Should other investigators wish more detailed information on this model, they are urged to contact LAIR.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6911	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061102BT1R	04	147
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Bone Repair (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 03	74 03	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	5	59
	CURRENT 74	7	30

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
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ADDRESS:

RESPONSIBLE INDIVIDUAL

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TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Maxillofacial Sciences Division

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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TELEPHONE: 415-561-4042

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Payne, T.F., CPT DC

NAME: Lilly, G.E., COL DC DA

21. GENERAL USE

22. KEYWORDS (Precede each with Security Classification Code) (U) fractures

(U) avulsion wound; (U) bone repair; (U) radioisotopes; (U) mandibular bone grafts

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To determine reliable quantitative and qualitative methods for assaying bone repair, and to further develop more objective laboratory and clinical endpoints for examining and evaluating bone healing in order to reduce hospitalization time associated with bone injuries in military patients and to more accurately determine effects of different forms of therapy.

24. (U) Laboratory studies on dogs relating radioisotope activity, microscopic, clinical, radiographic and physical strength findings.

25. (U) 72 07 - 73 06 Laboratory studies on dogs have been accomplished and a reliable surgical model employing trephine defects in the tibia has been developed. Comparative physical strength determinations in this model have been conducted on three different type of graft materials. Physical strength determinations in this model have been reproducible and sufficiently sensitive to detect differences in strength of union between various graft materials and host bone. The trends reflected in the data plots appear to correlate well with physiologic processes as studied by histologic and morphologic examinations.

* Available to contractors upon originator's approval.

BONE REPAIR

Captain Richard C. Robert, Jr., DC
Major Thomas F. Payne, DC
Major Thomas R. Tempel, DC
Colonel Gilbert E. Lilly, DC
SP5 Kenneth D. Freeze

PROBLEM:

Accepted treatment for fractures and bone grafts of the jaws usually involves wiring the jaws together. In the military, the patient is hospitalized until bone repair has progressed to the point that the wires can be removed, the patient can eat a normal diet, and he is not in danger of aspirating foreign material into the lungs. Such care requires prolonged hospitalization and results in many man-days lost. Currently, the most reliable clinical criteria for determining adequacy of bone repair are physical manipulation, and, in the laboratory, microscopic evaluation. Both of these methods are subjective.

This study is being undertaken to determine more reliable quantitative and qualitative methods for assaying bone repair to develop more objective laboratory and clinical endpoints for studying and evaluating bone healing.

APPROACH:

A model was developed for comparing union strength between various bone graft materials and recipient bone. It became obvious early in our investigations that such a model would have to satisfy several rigid criteria: 1) Uniform surgical defects and grafts to enable comparisons from graft site to graft site and animal to animal; 2) Efficient utilization of experimental animals to yield the maximum amount of data from each animal without compromising the healing process; and 3) Accurate, reproducible measurements of strength which maintain their validity under statistical analysis.

The surgical model which appeared to best satisfy these criteria was the placement of cylindrical graft plugs in trephinations along the medial surface of the

tibia in dogs. Allogeneic donor plugs were cut from cadaveric dog tibias with a 6 mm internal diameter trephine under irrigation with chilled normal saline. Autologous plugs were similarly obtained from the radius of the recipient animal. The superior and inferior surfaces of each plug were flattened and the thickness of the plug measured and recorded. Recipient sites for the graft plugs were prepared by drilling holes in the host tibia with a machinist's bit of the same diameter as the graft plug. This procedure provided exact approximation of graft and host bone along their mutual interface.

Using the procedures described above, a study was undertaken to compare healing in four recipient sites. These were: (1) allogeneic non-decalcified bone, (2) unfilled defect, (3) autologous bone from the radius and (4) SDAB or surface decalcified allogeneic bone. The SDAB plugs were defatted in 1:1 chloroform-methanol and decalcified in 0.6N hydrochloric acid for 6 hours, as described in work Unit No. 064, "Mandibular Bone Grafts".

Post-surgical sacrifice intervals were 2,3,4,6, and 8 weeks. The defects were prepared bilaterally in the tibias of three dogs per sacrifice interval. At the second, fourth, sixth and eighth week, a fourth dog was added for histological examination and photographic documentation. Hence, our data are based on a total of 152 graft sites prepared in 19 dogs.

After sacrifice, the bones were cut into sections which were mounted for physical strength testing. A device was constructed to position specimens on an Instron Universal Testing Apparatus located at the Dental Research facility of the United States Public Health Service Hospital in San Francisco, California. Strength of graft union was determined by measuring the force required to shear the healing interface between the graft and surrounding host bone. Shearing was accomplished by a machine punch under static loading at 0.2 cm per minute. The shearing force was divided by the surface area of interface between graft and recipient bone to obtain force per unit area.

RESULTS:

The shearing strength data recorded in kg/cm^2 were plotted against time in weeks to obtain time-strength curves for comparisons. (see Figure) The data plots were subjected to a two-way analysis of variance with replication and were found to be different at significance levels of less than 0.0005. A multiple comparison analysis according to Tukey was performed to enable individual comparisons with an overall probability of error at the 0.05 level. All four recipient sites showed a significant increase in strength at the sixth week as compared to the second week. Autologous grafts had strengths significantly greater than SDAB grafts from the third to the eight week. At the sixth and eight week, autologous and allogeneic strengths were statistically equal and both were significantly greater than that of SDAB.

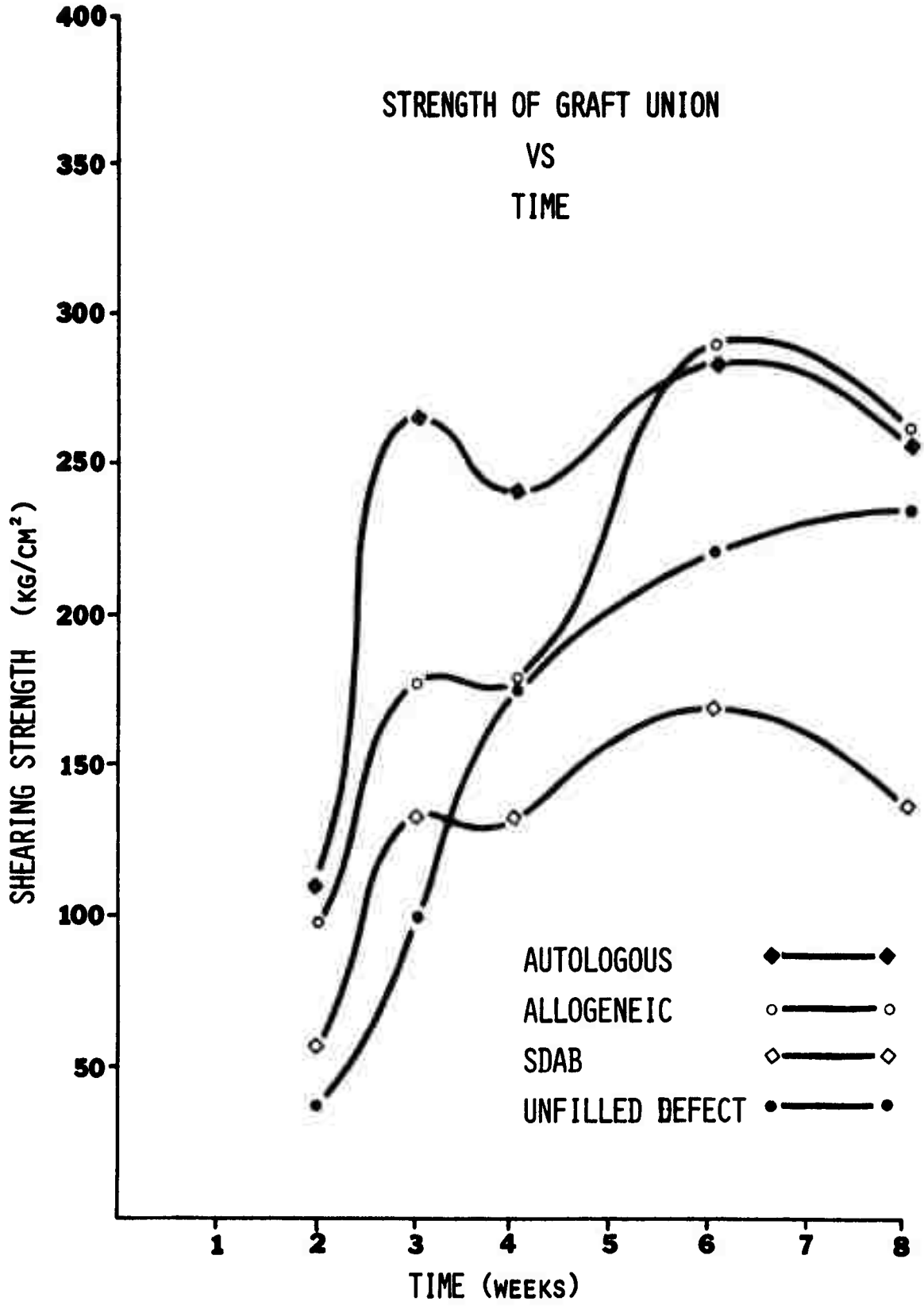
DISCUSSION:

The three graft materials yielded double-peaked curves as compared to the sigmoid curvature of the plot for the unfilled defect. We ascribe the first peak of the graft curves to the marrow-vascular component of the healing process as evidenced by gross observation of the extent and density of endosteal proliferation in longitudinal sections made at the third week. The medullary bone fill exhibited great homogeneity and minimal porosity. However, sections at the fourth and sixth weeks indicated that the endosteal proliferation had undergone considerable resorption and was far less dense. There was increased porosity and irregularity of the medullary component at this time.

The second peak of the graft curves, occurring at the sixth week, appeared to correspond to the cortical component of healing. This peak was followed by a dip at eight weeks which is assumed to reflect the resorption and revascularization of the graft plug as was evidenced in histologic slides.

FUTURE PLANS:

As remodeling continues, the strength of graft union should increase to that of normal bone, which is approximately 600 kg/cm^2 in the dog. A continuation of this study to the fourteenth postoperative week is in progress.


STRENGTH OF GRAFT UNION
VS
TIME
SHEARING STRENGTH (KG/CM²)
400
350
300
250
200
150
100
50
1
2
3
4
5
6
7
8
TIME (WEEKS)
AUTOLOGOUS
ALLOGENEIC
SDAB
UNFILLED DEFECT

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DA OA 6920
2. DATE OF SUMMARY: 73 07 01
REPORT CONTROL SYMBOL: DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A061102A825	00	040
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Early Management of Oral and Maxillofacial Wounds (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 - Clinical Medicine; 012900 - Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 11	74 06	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PREVIOUS 73	11	57
FISCAL YEAR CURRENT 74	10	50

19. RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Institute of Research
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RESPONSIBLE INDIVIDUAL
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TELEPHONE: 415-561-5181

21. GENERAL USE

20. PERFORMING ORGANIZATION
NAME: Letterman Army Institute of Research
Maxillofacial Sciences Division
ADDRESS: Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Lilly, G.E., COL DC
TELEPHONE: 415-561-5160
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Cutcher, J.L., LTC DC
NAME: Payne, T.F., MAJ DC DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) bone; (U) maxillofacial wounds; (U) wound incidence; (U) debridement; (U) surgery; (U) dentistry; (U) avulsion wounds; (U) sutures

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) 1) To establish nature, incidence, cause and management problems of oral and maxillofacial injuries occurring in Army populations; 2) To reduce individual morbidity and non-effectiveness and supportive care by developing methods and techniques for management of oral and maxillofacial wound.

24. (U) 1) Documentation of nature, incidence and cause of oral and maxillofacial injuries by survey of selected US Army treatment facilities in Vietnam; 2) Laboratory studies directed toward development of methods, which are practical under military field conditions; for management of gunshot wounds of the maxillofacial area; 3) Human clinical studies to validate laboratory studies.

25. (U) 72 07 - 73 06 Investigations are being conducted in eight sub-projects: Incidence of Oral and Maxillofacial Injuries; Early Restoration of Oral Integrity; Tissue Reaction to Sutures; Early Restoration of Mandibular Continuity; Mandibular Continuity; Mandibular Bone Grafts; Oral and Maxillofacial Wound Infections; Repair of Salivary Gland Ducts; Actinic Blocking Agents for Protection of Lip Mucosa.

See specific DD Form 1498 for current status of each sub-project.

* Available to contractors upon originator's approval.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6807	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	K. Complete	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	061
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Early Restoration of Oral Integrity (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
017100 - Weapons Effects; (U) 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 11	73 06	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	0	5
	CURRENT 74	0	0

19. RESPONSIBLE DOD ORGANIZATION
NAME:* Letterman Army Institute of Research
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TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION
NAME:* Letterman Army Institute of Research
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Lilly, G.E., COL DC
TELEPHONE 415-561-5160
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Cutcher, J.L., LTC DC
NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code) (U) combat; (U) dimethylsiloxane (U) wounds; (U) treatment; (U) oral; (U) maxillofacial; (U) weapons effects;

23. TECHNICAL OBJECTIVE.* 24. APPROACH; 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) When the body is injured by a blast or missle in military combat situations, variable amounts of soft tissue may be lost (avulsed). This study is directed towards developing methods of management to reduce the following effects of maxillofacial wounds: 1) Facial deformity; 2) Physical and psychological complications; 3) Requirements for special supportive care; 4) Lost duty time.

24. (U) Clinical studies on early restoration of selected orofacial wounds in a combat theater with RTV 382 dimethysiloxane immediate protheses. Laboratory studies on methods for delayed primary closure of oral wounds to reduce the incidence of wound breakdown.

25. (U) 72 07 - 73 06 A synthetic material has been used in human battlefield casualties to temporarily replace missing soft tissue of the maxillofacial area. Findings indicate that this material and method of management are effective in the treatment of large maxillofacial avulsion wounds. No evidence of untoward reaction has been found. Due to the disengagement of U.S. Forces in Southeast Asia, no battlefield casualties have been treated with this device during this fiscal year. The results of this study have been promulgated in the professional community: Salem, J.E., Lilly, G.E., Thompson, C.W., Prosthesis for Maxillofacial Avulsion Wounds: A Clinical Trial in Vietnam. J Trauma, 12:501-508, June 1972.

*Available to contractors upon originator's approval.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6809	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	062
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Incidence of Oral and Maxillofacial Injuries (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 09	74 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	2	2.5
	CURRENT 74	2	10

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

ADDRESS:*

RESPONSIBLE INDIVIDUAL

NAME: Akers, W.A., COL MC

TELEPHONE: 415-561-5181

21. GENERAL USE

20. PERFORMING ORGANIZATION

NAME:* Letterman Army Institute of Research
Maxillofacial Sciences Division

ADDRESS:* Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME:* Lilly, G.E., COL DC

TELEPHONE: 415-561-4042

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Rael, E.M., DAC

NAME: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) oral; (U) maxillofacial; (U) wounds; (U) injuries; (U) incidence; (U) treatment

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) This investigation was undertaken to obtain information concerning the relative incidence and types of maxillofacial injuries treated by the Federal Dental Services. This information is considered essential for determination of the Federal Dental Services' commitment in this area which may be used as a guide for staffing, support required, orientation of training programs and direction of research activities.

24. (U) Survey of maxillofacial wounds at selected overseas installations in the Republic of Vietnam. Reports are submitted by attending Army oral surgeons. Data are tabulated and analyzed by electronic data processing equipment.

25. (U) 72 07 - 73 06 To date, over 6,000 cases of maxillofacial injuries have been reported. Preliminary tabulation has been accomplished on 1800 of these cases. Computer programing is currently being accomplished to allow more complete analysis and correlation of these data.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

Incidence of Oral and Maxillofacial Injuries

Colonel Gilbert E. Lilly, DC
Miss Erlinda Rael, DAC

PROBLEM:

Previous studies conducted by this division, based on data on over 10,000 oral and maxillofacial injuries in Vietnam, revealed that the incidence of such injuries is much higher than previously reported (as high as 24 percent of all admissions for trauma at one major hospital reporting over 1100 maxillofacial injuries in a one year period). In addition, the nature of these injuries and resulting complications required more sophisticated treatment and supportive care than that traditionally associated with oral and maxillofacial injuries. These findings indicated: a) a greater dental commitment than previously realized; b) a requirement for greater support by the Medical Department; c) the need for more extensive training in certain areas; d) a requirement for redirection of research programs to deal with identified problems.

Current methods of reporting injuries incurred in the Federal Services are not sufficiently detailed to provide identifiable and usable data on maxillofacial injuries. Information on the type, number, treatment, and hospitalization time associated with maxillofacial injuries occurring within the United States is needed, since the majority of the population serviced by the Federal Dental Services is located within the United States. Such information is necessary to determine the Federal Dental Services' commitment in this area, which may be used as a guide for staffing, support required, orientation of training programs and direction of research activities.

The purpose of this study is to determine: 1) The commitments of the Federal Dental Services to the management of maxillofacial injuries and to establish the support required to meet these commitments. 2) The number and type of maxillofacial injuries at selected Federal hospitals. 3) The nature of maxillofacial injuries, the treatment required, complications associated with such injuries and identification of specific

problem areas.

APPROACH:

The oral surgery services at selected hospitals of the Air Force, Army, Navy, Public Health Service, and Veterans Administration are furnished survey forms to be completed on all cases of maxillofacial trauma treated at their respective facilities. A representative from each service has been delegated as a coordinator to collect and review the data and forward it to Maxillofacial Sciences Division, Letterman Army Institute of Research. The data is processed and tabulated by electronic data processing equipment. Accrued information is made available to the Chiefs of the Federal Dental Services and, upon their approval, promulgated in the usual manner.

RESULTS:

To date, over 6,000 cases have been reported and the forms returned to LAIR. Data on these have been transcribed to key punch cards.

A preliminary tabulation of the data received during the last six months of FY 72 has been accomplished and is presented in tabular form at the conclusion of this report. A total of 1811 cases of maxillofacial injury were reported during this period and are the basis for this tabulation. In general, the data presented is only a simple tabulation. Although numerous internal comparisons are possible, this has not been accomplished at this time. Such comparisons are currently being accomplished and will be the subject of more detailed reports to each of the specific services concerning the data relative to their service.

A total of 86 cases were reported by Air Force installations (4.8 percent of the total), 1083 were received from Army installations (59.8 percent of the total), 406 were from USPHS facilities (22.4 percent of the total), and in 6 cases (0.3 percent), the reporting service was not specified. No reports were received from the Veterans Administration. The data reported for naval facilities represents only the last two months of FY 72.

The patients ranged in age from 1 to 89 years; however, 82.2 percent were in the first three decades of life. Slightly over 78 percent of the patients were males.

DISCUSSION:

Although data collection was to start 1 January 1972, delays in initiating the study were encountered in some areas. These problems have in most cases been resolved and, as FY 1973 draws to a close, it is hoped that all services and hospitals selected will be actively participating in this study.

Data collection and submission have been accomplished primarily by the dental branches of each of the federal services. This bias must be recognized in evaluating the data collected to date. It is hoped that in the future, others engaged in managing oral and maxillofacial injuries will actively participate in the survey so that the data will be more representative of the workload and commitments of each of the federal services and hospitals in the treatment of oral and maxillofacial injuries.

FUTURE PLANS:

Computer programing of these data is currently being accomplished. This should be completed within the next three months and should make prompt tabulation and more detailed analysis of these data possible during FY 74.

DISPOSITION OF PATIENTS

Duty	687 (37.9)
Transfered	42 (2.3)
Released to Own Care	880 (48.6)
Died	5 (0.3)
Other	146 (8.1)
Not Specified	51 (2.8)
TOTAL	1811

() Percent of total.

NUMBER OF OUTPATIENTS AND NUMBER OF DAYS TREATED AS AN INPATIENT

Outpatient	985 (54.4)
Inpatient	
1-10	217 (12.0)
11-20	222 (12.3)
21-30	72 (4.0)
31-40	94 (5.1)
41-50	122 (6.7)
51-75	35 (1.9)
76-100	9 (0.5)
101 +	12 (0.7)
Not Specified	43 (2.4)
TOTAL	1811

() Percent of total cases treated.

HOSPITAL SERVICE RESPONSIBLE FOR PATIENT

Oral Surgery	1035 (57.2)
Plastic Surgery	27 (1.5)
Otolaryngology	53 (2.9)
Ophthalmology	16 (0.8)
General Surgery	197 (10.9)
Other	478 (26.4)
Not Specified	5 (0.3)
TOTAL	1811

() Percent of total.

SOFT TISSUE INJURIES

	INTRAORAL	EXTRAORAL
Lacerated	522 (28.8)	859 (47.4)
Contused/Abraded Hematoma	221 (12.2)	395 (21.8)
Avulsed	32 (1.8)	29 (1.6)
Treated by Other Primarily	169 (22.4)*	702 (70.1)
Primarily Oral Surgeon	587 (77.6)*	299 (29.9)*

() Percent of total number of patients.
* Percent of patients treated with a soft tissue injury.

FRACTURE TREATMENT

	MANDIBLE	MAXILLA	ZYGOMA	ORBITAL FLOOR
Closed Reduction	307 (68.2)	44 (43.1)	11 (9.3)	7 (10.6)
Intraoral Open Reduction	39 (8.7)	17 (16.7)	17 (14.4)	10 (15.2)
Extraoral Open Reduction	60 (13.3)	24 (23.5)	48 (40.7)	26 (39.4)
Not Specified	44 (9.8)	17 (16.7)	42 (35.6)	23 (34.8)
Total	450	102	118	66
Primary Oral Surgeon	391 (86.9)	74 (72.5)	54 (45.8)	28 (42.4)

() Percent of fractures of specific bone indicated.

MAXILLOFACIAL FRACTURES

	MANDIBLE	MAXILLA	ZYGOMA	ORBITAL FLOOR
Number	450 (24.8)[1]	102 (5.6)[1]	118 (6.5)[1]	66 (3.6)[1]
Comminuted	67 (14.9)[2]	32 (31.4)[2]	18 (17.6)[2]	21 (31.8)[2]
Avulsed	15 (3.3)[2]	3 (2.9)[2]	1 (0.8)[2]	4 (6.1)[2]
Alveolar Process only	11 (24.4)[2]	17 (16.7)[2]	-	-

[1] Percent of total cases reported.
[2] Percent of fractures for indicated bone.

CIRCUMSTANCES ASSOCIATED WITH INJURY

Altercation	584 (32.2)
Auto	286 (15.8)
Auto (Ped)	14 (0.8)
Motorcycle	37 (2.0)
Motorcycle (Ped)	3 (0.2)
Other Vehicle	37 (2.0)
Gunshot	9 (0.5)
Other	814 (45.0)
Not Specified	27 (1.5)
TOTAL	1811

COMPLICATIONS OF BONE HEALING

	NUMBER	PERCENT OF TOTAL CASES
Delayed Union	14	0.77
Malunion	5	0.28
Nonunion	8	0.44

OPERATING ROOM AND GENERAL ANESTHESIA

Operating Room	267 (14.7)
General Anesthetic	250 (13.8)
Total Cases	1811

() Percent of total.

COMPLICATING FACTORS IN MANAGEMENT

	Number Reported	Percent of Total Patients
CNS	60	3.3
Medical Risk	40	2.2
Infection	43	2.4
Operative/Postop Complication	32	1.8

MAJOR INJURY

	Number	Percent of Cases
Maxillofacial	1594	88.0
Other	115	6.4
Indeterminate	47	2.6
Not Specified	55	3.0

INJURED TEETH & TRACHEOSTOMY

	Number Reported	Percent of Total Patients
Teeth Injured/Avulsed	541	29.9
Tracheostomy	32	1.8

OTHER SIGNIFICANT INJURY

	Number Reported	Percent of Total Cases
Extremities	178	9.8
Trunk	78	4.3

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6809	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	K. Complete	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110AB15	00	003
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Early Restoration of Mandibular Continuity (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine; 017100 - Weapons Effects

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 06	73 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	.1	9.0
	CURRENT 74	0	0

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

NAME: Akers, W.A., COL MC

TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Maxillofacial Sciences Division
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Lilly, G.E., COL DC

TELEPHONE: 415-561-4042

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Cutcher, J.L., LTC DC

NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) osseous avulsion wounds
(U) maxillofacial; (U) weapons effects; (U) early treatment; (U) mandible; (U) oral;

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Wounds of the face due to missiles or blast may result in losing large portions of the lower jaw bone (mandible). When large portions of the mandible are lost, the overlying soft tissues collapse. The patient is grossly disfigured and experiences extreme difficulty in eating, breathing, and talking. Studies are directed toward methods of management which will reduce morbidity associated with such wounds by (1) Early temporary restoration of mandibular continuity to permit support of soft tissue in a more normal anatomic position, and (2) earlier replacement of lost bone with bone graft.

24. (U) Clinical studies in the combat theater of operations in Vietnam. Laboratory studies on permanent replacement of missing portions of the mandible with bone grafts.

25. (U) 72 07 - 73 06 Laboratory and clinical studies have indicated that mandibular continuity can be effectively temporarily restored, and soft tissues supported, with preformed silicone segments. Preformed silicone mandibles have been fabricated and evaluated in clinical trials.

* Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

Early Restoration of Mandibular Continuity

Colonel Gilbert E. Lilly, DC
Lieutenant Colonel James L. Cutcher, DC
Colonel Calvin Thompson, DC

PROBLEM:

Maxillofacial gunshot injuries frequently result in loss of major portions of the mandible. Such injuries, particularly when they involve the anterior part of the mandible, result in collapse of the lower portion of the face due to lack of osseous support for the overlying soft tissues. These injuries are difficult to reconstruct and frequently result in major, permanent, facial disfigurement. The Letterman Army Institute of Research has been conducting studies directed toward developing more effective methods for managing such mandibular injuries in order to reduce the extent of soft tissue collapse.

APPROACH:

In cooperation with the Dow Corning Center for Aid to Medical Research, this work has resulted in the development of a preformed anatomic silicone mandible fabricated around a central noncorrosive metal strut for reinforcement. This silicone device is autoclavable and can be cut with a knife and prepared at surgery to restore any missing portion of the mandible from condyle to condyle.

RESULTS:

Studies on dogs have indicated that such a device is well tolerated by the tissues, is easily prepared to fit the osseous defect, and effectively supports the soft tissues.

Since its development and subsequent availability, operational requirements in Vietnam have not necessitated the use of this device on any battlefield casualties. It has, however, been successfully used on three patients with large benign neoplasms of the mandible requiring resection of major portions of the bone. In these cases, the contour of the mandible was immediately restored at the time of resection with a segment of

preformed silicone mandible. The metal strut was embedded in the medullary stump of the mandible and additional stabilization was afforded by stainless steel wires. Intermaxillary fixation was maintained postoperatively. Preliminary findings on these three patients indicate that this device: a) is well tolerated with no untoward reactions observed; b) effectively supports the soft tissues; c) is easily adapted to the osseous defect.

DISCUSSION:

This device and method of management is not intended to be a permanent, functional replacement. Rather, it is a temporary method for supporting the tissues and maintaining a space for bone grafting at a later date.

Other investigations have reported the use of a mandibular prosthesis fabricated from blocks of silicone and Kirschner wires by the surgeon in the surgical treatment of advanced oral cancer. A preformed mandible such as the one described in this communication could conceivably simplify the procedure described by other investigators.

FUTURE PLANS:

This study is being completed. Follow-up of clinical cases will continue.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6909	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	064
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Mandibular Bone Grafts (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 03	74 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PREVIOUS 73	4	20
FISCAL YEAR CURRENT 74	4	11

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Letterman Army Institute of Research
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RESPONSIBLE INDIVIDUAL

NAME: Akers, W.A., COL MC

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20. PERFORMING ORGANIZATION

NAME:* Letterman Army Institute of Research
Maxillofacial Sciences Division

ADDRESS:* Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME:* Lilly, G.E., COL DC

TELEPHONE: 415-561-4042

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Cutcher, J.L., LTC DC

NAME: Hourigan, M., LTC DC DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) mandibular bone grafts; (U) maxillofacial; (U) bone induction; (U) avulsion wounds

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To develop methods to reduce reconstruction and hospitalization time currently associated with mandibular bone grafts in military patients. Current patients needing mandibular grafts require an average 509 days of hospitalization, and are discharged with 25 percent permanent disability.

24. (U) Laboratory studies on dogs evaluating the relative suitability of surface decalcified lypholized mandibular bone grafts obtained from other dogs and marrow bone grafts from the same animals.

25. (U) 72 07 - 73 06 To assess the efficacy of ethylene oxide gas as a sterilizing agent for surface decalcified allogenic canine bone (to be used as canine mandibular graft material), a modified decalcification procedure has been developed. Preliminary bacteriologic findings are insufficient for interpretation. Extensive pilot testing indicates, however, that use of gaseous ethylene oxide following chemical processing of such bone may be a superior alterative to use of high levels of ionizing radiation for sterilization of this material.

*Available to contractors upon originator's approval.

Mandibular Bone Grafts

Colonel Gilbert E. Lilly, DC
Lieutenant Colonel James L. Cutcher, DC
Lieutenant Colonel Matthias J. Hourigan, DC

PROBLEM:

A survey of 9,439 patients with maxillofacial injuries received in Vietnam revealed that in 9.4 percent of patients with mandibular fractures due to hostile action, avulsion of a significant portion of the mandible occurred.

Many of these patients required bone grafts to restore the mandibles. A review of 89 patients who required such mandibular bone grafts at selected Army hospitals revealed that the hospitalization time for these patients averaged 509 days, with a range of 138 to 1,392 days. The patients in this group who were discharged from the military service had an average of 25 percent disability related to their mandibular wounds. Graft failures, or complications requiring additional grafts, occurred in 12.4 percent of these patients. These findings indicated the continued need for research directed toward reducing the morbidity and hospitalization time associated with mandibular bone grafts.

Previous studies on dogs have shown the use of surface decalcified allogenic bone (SDAB) as a "crib" supporting a graft of autologous hematopoietic bone marrow, has certain advantages over an earlier, similar technique employing a vitallium mesh framework with a micropore filter to support the marrow material. The metal framework is difficult to adapt at operation and may require surgical removal after the graft has healed. In contrast, SDAB can be easily trimmed and adapted to the graft site and is eventually resorbed and replaced by new bone following the grafting procedure. Moreover, mandibular grafts employing SDAB have thus far exhibited no evidence of host rejection nor inhibition of host osteogenic activity at the graft site.

This study was undertaken to determine the feasibility of using SDAB for mandibular bone grafts and, if feasible, to develop methods for accomplishing such

grafts.

APPROACH:

The probability of contaminating the SDAB graft material during its surgical procurement and subsequent processing has necessitated the search for a suitable means of sterilizing this material without compromising its potential for grafting success. Although ionizing radiation (2,500,000 rads) has been used for sterilization by some investigators, there is a question concerning the effect of such a radiation level on the so-called "bone induction principle" - a collagenous substance possibly involved in osteogenesis at the graft site.

Use of "cold sterilizing" solutions consisting of various mixtures of antibiotics in saline, as advocated by certain investigators, has not been effective in this laboratory.

In an effort to find a simple, inexpensive method for sterilizing SDAB, this laboratory is currently evaluating the efficacy of ethylene oxide gas. Towards this end, bacteriologic analysis is being incorporated into the following bone surface decalcification procedure:

1) Bone is removed from the canine tibia and stored at 0-5°C in sterile normal saline.

2) Bone is cut into 32 fragments (approximately 0.5 cm^3) on a band saw. At this point the bone can be considered to be contaminated.

3) Bone is placed in a sterile beaker containing 300 ml chloroform and 300 ml absolute methanol and stirred 4 hours at 0-5°C.

4) The solution is decanted and step 3 is repeated for 4 hours and once again for 16 hours.

5) Bone is washed in absolute methanol for 30 minutes to remove residual chloroform.

6) Bone is surface decalcified for 6 hours in 0.6N HCl made up in 1.0 N NaCl.

7) Bone is carried through a series of 7 washes to remove residual acidity. Sterile normal saline (S) and 70 percent ethanol (E) are employed at 0-5°C in the following sequence: S,E,E,S,S,S,S. Each wash runs 30 minutes, with stirring.

8) Bone is washed for 2 hours at 0-5°C in 95 percent ethanol, placed in a laminar flow filter hood and dried with sterile filtered air for 2 hours.

9) Bone is placed in an ethylene oxide gas sterilizer (Bard Model 2270) for 24 hours at 25°C. It is then aerated for 12 hours at 25°C.

10) Bone is lyophilized aseptically for 18 hours.

11) Bone is aseptically transferred to individual sterile storage bottles, capped, and placed in a refrigerated desiccator at 0-5°C until used for grafting procedures. (Note: Step 11 has not as yet been implemented since all bone segments utilized in this procedure to date have been used for bacteriologic analysis.)

Bacteriologic evaluation of donor bone segments is performed immediately following steps 2,8,9 and 10. Eight individual bone segments are removed from the process and analyzed at each of these 4 stages as follows: 4 segments are cultured in brain heart infusion broth; 4 segments are cultured in fluid thioglycollate medium. Of the resulting 8 culture tubes incubated at 37°C, 4 are incubated anaerobically (95 percent hydrogen, 5 percent CO_2) and 4 are grown in an atmosphere of 4 percent CO_2. Culture tubes are checked daily for 3 weeks before discarding. Positive cultures are identified generically and speciated where feasible.

RESULTS:

The methodology described in the previous section is the result of extensive pilot studies. Preliminary findings based upon one definitive bacteriologic analysis of 32 bone segments processed as outlined are as follows:

STEP 2: Positive bacterial growth (Staphylococcus aureus, Group D streptococci and Lactobacillus species).

STEPS 8, 9 and 10: No growth.

DISCUSSION:

The preliminary nature of these findings precludes interpretation of their validity. It became apparent, however, in the course of several experiments preceding the development of the current procedure, that the chemical solutions employed in decalcification inhibit bacterial growth on SDAB. Whether this will be consistently true remains to be seen. Fresh solutions are used for all processing.

Ethylene oxide gas is known to be ineffective against desiccated spores and many dehydrated vegetative bacterial cells. Conversely, the presence of water or saline in the bone sample to be sterilized is known to effectively block penetration of the gas into the Haversian systems of bone. For this reason, the introduction of a 2 hour 95 percent ethanol wash (followed by a 2 hour air-drying procedure) into the methodology was deemed necessary. In order to preclude vacuum desiccation of viable, residual bacteria following completion of surface decalcification, gas sterilization is now being performed prior to lyophilization. We believe that this modified sequential procedure, coupled with stringent aseptic technique, shows promise of offering an effective method for sterilizing SDAB.

FUTURE PLANS:

Additional studies are in progress to assess the efficacy of ethylene oxide gas as a sterilizing agent for SDAB. Bone samples adjudged to be so sterilized will then be evaluated in grafting procedures.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6811	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	K. Complete	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3A062110A825	00	045
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Tissue Reaction to Sutures (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 - Clinical Medicine; 012900 - Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 06		DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	.5	5.0
	CURRENT 74	0	0

19. RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Institute of Research
ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL
NAME: Akers, W.A., COL MC
TELEPHONE: 415-561-5181

21. GENERAL USE

20. PERFORMING ORGANIZATION
NAME: Letterman Army Institute of Research
ADDRESS: Maxillofacial Sciences Division
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Lilly, G.E., COL DC
TELEPHONE: 415-561-5160
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Cutcher, J.L., LTC DC
NAME: DA

22. KEYWORDS (Precede each with Security Classification Code) (U) Maxillofacial wounds; (U) sutures; (U) tissue reaction; (U) oral surgery; (U) mouth; (U) mucous membranes

23. TECHNICAL OBJECTIVE 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To determine which suture materials are most acceptable for use in the oral cavity. Patients who receive maxillofacial wounds in the military frequently require the prolonged retention of sutures. Under such conditions the relative acceptability of the suture can become a critical factor, resulting in wound breakdown, if inappropriate sutures are used.

24. (U) Laboratory studies in dogs evaluating the histologic response of oral tissues to various suture materials and clinical studies in humans to verify laboratory findings.

25. (U) 72 07 - 73 06 The response of the oral tissue to 18 different suture materials has been evaluated in animal, clinical and laboratory studies. The monofilament suture materials and the multifilament resorbable suture material, polyglycolic acid, were associated with milder tissue responses than the nonresorbable multifilament suture materials studied. The physical nature and biocompatibility of polyglycolic acid sutures make them the suture of choice for intraoral wound closure where prolonged retention of sutures is likely. Within the past year no new suture materials have become available for evaluation.

* Applicable if publication anticipated.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6920	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	H. Terminate	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	067
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Repair of Salivary Gland Ducts (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 08	73 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	0	4.5
	CURRENT 74	0	0

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

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RESPONSIBLE INDIVIDUAL

NAME: Akers, W.A., COL MC

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20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Maxillofacial Sciences Division

ADDRESS: Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Lilly, G.E., COL DC

TELEPHONE: 415-561-4042

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) combat surgery; (U) oral injuries; (U) salivary gland duct repair

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) This study is directed towards developing more effective methods for managing gunshot wounds of the maxillofacial area which involve injury to major salivary gland ducts. Objectives: (a) To reduce individual morbidity and hospitalization time associated with such injuries; (b) Promote normal repair and function of major salivary glands associated with such injuries.

24. (U) Pilot studies on dogs evaluating various methods for repair of salivary gland ducts and determining mode of procedure that will allow normal salivary drainage, promote gland repair and function and prevent formation of salivary fistula on the face.

25. (U) 72 07 - 73 06 Due to budgetary and personnel limitations and the higher priority of other research, no investigations have been conducted in this area. This project is being terminated.

*Available to contractors upon originator's approval.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6923	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	068
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Oral and Maxillofacial Wound Infection (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 08	74 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PREVIOUS 73	4	10.5
CURRENT 74	3.5	20

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Letterman Army Institute of Research

ADDRESS:* Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

NAME: Akers, W.A., COL MC

TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION

NAME:* Letterman Army Institute of Research

ADDRESS:* Maxillofacial Sciences Division
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME:* Cutcher, J. L., LTC DC

TELEPHONE: 415-561-5160

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) wound infection; (U) antibiotic susceptibility testing; (U) pathogenic bacteria

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) a. To reduce individual morbidity and mortality associated with oral and maxillofacial wound infections; b. To determine the most effective methods for treating infected oral and maxillofacial wounds.

24. (U) Clinical and laboratory study of infected human oral and maxillofacial wounds, identifying all mocrobial genera cultured from such infections, and delineating the antibiotic susceptibility patterns of all microorganisms involved. Such information is collated with the clinical course of infection in order to evaluate the effect of drug therapy.

25. (U) 72 07 - 73 06 A total of 156 infection samples have been analyzed from 82 patients to date. From this group, 555 pure culture strains of bacteria have been grown, reflecting 40 different species (17 gram positive, 23 gram negative). The total number of penicillin-resistant strains (PRS) grown was 175/155 (31.5 percent). Gram positive PRS comprised 102/175 (58.2 percent) of these strains. In 75 infection samples obtained from 47 patients not receiving antibiotics at culture, 42/75 (56 percent) contained PRS. Of these 42 samples, 32 (74.7 percent) contained gram positive PRS. These findings emphasize the need for a continuing appraisal of the microfloras of oral and maxillofacial infections. Knowledge of changing antibiotic susceptibility patterns of involved bacteria constitutes a primary clinical guideline for therapy in cases where the empirical use of antibiotics is required.

* Available to contractors upon originator's approval.

ORAL AND MAXILLOFACIAL WOUND INFECTION

Lieutenant Colonel James L. Cutcher, DC
Judith B. Richey, B.S.
Arthur Z. Tanner, B.S.
Colonel Donald B. Osbon, DC

PROBLEM:

The empirical use of antibiotics as an initial approach to therapy of oral and maxillofacial infections requires a knowledge of the surgical flora of the anatomic region as well as the antibiotic susceptibility patterns of organisms frequently implicated in such infections.

Penicillin has long been advocated as the drug of choice in many oral and maxillofacial infections. In the past 10 years, however, evidence has accumulated which suggests that the "traditional" infection microbiota is changing. Such flora changes would necessarily entail a reconsideration of therapeutic management methods, especially in cases of empirical or prophylactic administration of antibiotics.

In contrast to other surgical disciplines, where extensive literature reports provide a substantial basis for infection therapy, criteria for use of antibiotics in oral and maxillofacial infections are not well defined. The complex nature of the involved flora is undoubtedly a major factor in this situation.

The purpose of this investigation is to survey the microfloras of oral and maxillofacial infections and to establish guidelines for therapy by determining microbial population characteristics and antibiotic susceptibility patterns of bacteria cultured from these infections.

APPROACH:

This study will be performed on selected patients having oral and maxillofacial infections, who present for treatment at the Department of Dentistry, Letterman Army Medical Center.

Prior to definitive therapeutic measures, a bacteriologic sample will be obtained from the inflammatory site and inoculated directly into fluid thioglycollate medium. A dry culture swab sample will also be obtained. Both specimen swabs, accompanied by pertinent data relating to the patient and the lesion will be immediately forwarded to the Oral Microbiology Laboratory, Maxillo-facial Sciences Division, Letterman Army Institute of Research.

Specimens will be evaluated for identification of aerobic, facultative and anaerobic microorganisms cultured from these lesions. Antibiotic susceptibility patterns, where feasible, will be determined according to the Kirby-Bauer disc diffusion method.

All laboratory findings relating to culture characterization and antibiotic susceptibility data will be collated with the clinical course of each infection. Additional specimens will be obtained and evaluated on individual patients as deemed necessary by the attending clinician.

Where possible, the presence of penicillin-resistant strains (PRS) will be evaluated on the basis of the therapeutic status of the patient at the time the infection sample was obtained. Thus, the incidence of gram positive and gram negative PRS in these infections can be related to the type of antibiotic therapy, if any, that the patient was receiving at the time of culture.

RESULTS:

A total of 156 infection samples have been obtained from 82 patients to date. From this population, 555 pure culture strains of bacteria have been grown, reflecting 40 different species (17 gram positive, 23 gram negative). Of these 555 pure culture isolations, 127 (22.9 percent) were gram negative bacteria. This represents a slight drop from the incidence cited in the FY 72 report (24.7 percent).

The total number of PRS cultured was 175/555 (31.5 percent). Gram positive PRS comprised 102/175 (58.2 percent) of these strains.

Regardless of the antibiotic therapeutic status of these patients at time of culture, 107/156 infection samples contained one or more PRS, an incidence of 68.6 percent.

From the 156 infection samples, 116 samples were selected and divided into 2 groups, based upon antibiotic therapy. Group I (41 samples, 24 patients) were receiving penicillin at time of culture. Group II (75 samples, 47 patients) received no antibiotics at time of culture nor for one month previously. The remaining 40 samples represented patients whose therapeutic status at culture was unknown.

In Group I: 37/41 samples (90.2 percent) contained PRS; 28/41 samples (68.3 percent) contained gram positive PRS. In Group II: 39/75 samples (52 percent) contained PRS; 30/75 samples (40 percent) contained gram positive PRS. The overall incidence of PRS in Group I infection samples, as compared to Group II, was significantly higher ($P<.005$).

In the 75 bacteriologic samples obtained from the 47 patients not receiving antibiotics at time of culture, 42/75 (56 percent) contained bacteria resistant to penicillin. Of these 42 samples, 32 (74.7 percent) contained gram positive PRS. Thus, 32/75 infection samples (42.6 percent) obtained from patients not receiving antibiotics contained gram positive PRS.

DISCUSSION:

For a variety of reasons, culture and sensitivity testing is frequently not utilized by the clinician in the initial administration of antibiotics. In the absence of the specific information obtainable from in vitro testing, the clinician must utilize an empirical approach based in large part upon a knowledge of epidemiological data relating to the infections which he encounters.

Therefore, although currently controversial in many of its specific applications, the use of empirical or prophylactic approaches to antibiotic therapy in oral and maxillofacial infections is recognized as a valid, and at times, essential, factor in their management.

The general availability of various penicillins, however, has led to the widespread use, and abuse, of these drugs with resultant increases in resistance of bacteria formerly susceptible to this type of antibiotic.

In view of the widely promulgated operating principle that penicillin G is the unquestioned drug of choice in those oral and maxillofacial infections where an empirical approach is initially indicated, the data accrued in this study to date are of interest. While no attempt has been made as yet to analyze the PRS on the basis of classical concepts of pathogenicity, it must be recognized that any bacterial species cultured from a clinical infection should be regarded as potentially pathogenic.

In this study, penicillin therapy in oral and maxillofacial infections is apparently related to a marked increase in the presence of PRS. Of potentially greater concern is the incidence of gram positive PRS cultured from patients not receiving antibiotic therapy.

These findings emphasize the need for epidemiologic data as clinical guidelines for empirical use of antibiotics in the management of oral and maxillofacial infections, when circumstances dictate such an approach.

FUTURE PLANS:

Clinical and laboratory studies will be continued and completed in FY 74.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6922	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	K. Complete	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	069
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Actinic Blocking Agents for Protection of Lip Mucosa (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 09	74 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	.5	.5
	CURRENT 74	.5	4

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Letterman Army Institute of Research

ADDRESS:* Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

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TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION

NAME:* Letterman Army Institute of Research

ADDRESS:* Maxillofacial Sciences Division
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Lilly, G.E., COL DC

NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) actinic damage; (U) lip mucosa; (U) actinic blocking agents; human volunteers

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Military field operations frequently require prolonged exposure to the sun. Such exposure can result in damage to the lower lip. Such sunburns are painful and interfere with voice transmission. This study is designed to determine those agents or agent which are the most effective in protecting the lip.

24. (U) In vivo laboratory studies on human volunteers to evaluate the protective effect of commercially available agents when applied to the lips. A Xenon Solar Simulator will be used which emits an ultraviolet light spectrum similar to that received on the earch's surface. By using graded time selected exposures the actinic blocking agent which gives the most durable protection when applied to the lip will be ascertained.

25. (U) 72 07 - 73 06 Elevel commercially available actinic blocking agents were evaluated in human volunteers. Agents containing p-aminobenzoic acid were superior to all other agents in terms of effectiveness and duration of protection. No agent gave reliable protection three hours after application to the lip.

*Available to contractors upon originator's approval.

Actinic Blocking Agents for Protection of Lip Mucosa

Major Thomas F. Payne, DC
Colonel Gilbert E. Lilly, DC
Major Thomas R. Tempel, DC

PROBLEM:

Sunburn and tanning of the skin are caused by short-term exposure to the ultraviolet light (UVL) portion of the sun's spectrum. Acute exposure to UVL causes chapping, cracking and peeling of the lips. Chronic exposure to UVL is important in the development of skin and lip carcinoma. Because of the relationship between actinic radiation and lip carcinoma, patients who present with a sun damaged lip are advised to avoid excessive sunlight, wear wide-brim hats and use actinic blocking agents when in direct sunlight.

While information is available for agents designed for the skin, no data is available for lip mucosa. This study was undertaken to determine the duration of protection of commercially available actinic blocking agents on lip mucosa.

APPROACH:

The UVL source used in this study was a Xenon Solar Simulator. The Solar Simulator utilizes a Xenon lamp which emits a UV spectrum of 290-425 NM with a Shott WG 320 and a Corning 9863 filter. This spectrum closely simulates the solar UVL spectrum as received on the earth's surface. The duration of protection of the actinic blocking agents or sunscreens was evaluated by the response to a minimum erythema dose or M.E.D. An M.E.I. is defined as the minimum exposure time in seconds which will produce a well defined erythema at the test site. After determining the M.E.D. for each subject on the lip and volar surface of the forearm, 10 seconds were added to insure against false negatives.

Twenty-three human volunteers were used to test seven agents specifically designed for use on the lips and four agents designed for use on the skin. The lip agents tested were: Almay Sun Stick, Blistex, Lipkote, Lipsaver, RV Paba, SunStick and Weatherproofer. The agents tested which were designed primarily for use on

the skin were: A-Fil Cream, PreSun, R.V.P., and Sun-Guard. (For chemical classification of these agents, see Table.)

These agents were tested in the following manner: An agent was applied to the lips and to a site on the ventral surface of the forearm. After an interval of one or three hours, the subjects received an exposure of their individual M.E.D. + 10 seconds to a 5x20 mm area on the lower lip and forearm. Approximately 18 hours after exposure, the test sites were read as nonprotective (exhibiting erythema) or protective (no erythema). The time interval between application of an agent and exposure did not include a meal. The only instructions given to the subjects were that they were not to deliberately remove the agent. On the lip, the exposure site was alternated from one side to the other. At no time was an M.E.D. given to a previously exposed site until the mucosa had returned to clinical normality. Exposures were normally 7 days apart.

RESULTS:

The average lip M.E.D. was 71 seconds with a range of 70 to 80 seconds. The average M.E.D. for the ventral surface of the forearm was 88 seconds with a range of 70 to 130. The M.E.D. for lip mucosa was always less than the individual's skin M.E.D.

The duration of protection for one and three hours on lip and skin is summarized as follows:

one hour ------lip------37 percent protection*
one hour ------skin-----64 percent protection
three hours----lip------18 percent protection
three hours ---skin-----43 percent protection

* "Percent protection" is the average incidence of protection against erythema provided to test subjects by all agents evaluated.

The observed difference in protection on lip and skin was significant at the .05 level using Chi-Square analysis of discordant pairs.

The best performance by an agent was 71% and 48% protection, one and three hours, respectively, after

application on the lips. Comparison of the agents by active ingredients revealed that actinic blocking agents containing p-aminobenzoic acid (RV Paba and PreSun) gave superior protection on lip and skin.

The most noticeable difference between the response of lip and skin to M.E.D. exposures as given in this study was the sensitivity of lip mucosa. Unprotected skin can withstand a 3 M.E.D. dose without blistering while an exposure in the 1.5 M.E.D. range will cause blistering or cracking of the lip. Tanning was observed following positive exposures to the skin, however, tanning was not observed on the lip. There was no evidence of either increased or decreased reactivity of the lip mucosa to multiple exposures.

DISCUSSION:

To provide protection for lip mucosa against one M.E.D. plus 10 seconds exposure, an agent should be applied at least hourly. The performance of the agents designed specifically for use on lip and skin was not statistically different from that of agents designed for use on the lips alone. With the exception of PreSun, the agents designed for use on the skin were judged by the volunteers to be unacceptable because of their "feel" on the lips.

While intensity of exposure from the solar simulator was artificial, the UV spectrum was almost identical to that received on the earth's surface. An M.E.D. is the smallest exposure possible which gives a clinical endpoint. It is felt that this method of evaluating actinic blocking agents is as close as possible to physiologic conditions.

FUTURE PLANS:

This study has been completed. Based upon the data presented, RV Paba will be recommended for standardization at the Letterman Army Medical Center pharmacy as the most efficacious actinic blocking agent designed for use on the lips and this study will be published in the professional literature.

TABLE

I. P-aminobenzoic acid

1. PreSun*
2. RV Paba

II. Amyl p-dimethylaminobenzoate

1. Weatherproofer

III. Salicylates

1. Lipkote
2. Lipsaver
3. Blistex

IV. Cinnamates

1. Almay Sun Stick
2. Sun Guard

V. Digalloyl trioleate

1. SunStick

VI. Titanium Dioxide

1. A-Fil Cream*

VII. Red Petrolatum

1. R.V.P.*

* Agent designed for use on skin

TECHNICAL SUPPORT DIVISION

Program Element 6.11.02.A
Defense Research Sciences, Army

Project Number 3A061102B71P
Biochemistry

Task Area Number 01

TECHNICAL SUPPORT DIVISION

The Technical Support Division became operational in July 1972 to fill the gap created by the demise of the Clinical Research Support Division. By virtue of a support agreement with the Clinical Research Service, LAMC, continuity was afforded a number of clinical research projects through services of the Pho Gamma III Scintillation Camera and the fully staffed and equipped Biochemistry Laboratory.

A major project concerned studies on the use of intravenous glycerol for the management of cerebral edema. Results to date indicate both the ability of brain to metabolize glycerol in vivo and the superiority of glycerol over mannitol for the control of intracranial pressure.

A study of the pharmacokinetics of FANSIDAR, an anti-malarial drug consisting of a combination of sulfadoxine and pyrimethamine was initiated. The sulfadoxine assays on serum collected during a field trial of the drug in Malaysia have been completed and show a drug half-life of 215 $\pm$ 39 hours. A gas chromatographic method capable of measuring nanogram amounts of pyrimethamine in serum has been developed and early completion of the project is anticipated.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6812	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	K. Complete	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71P	01	091
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Clinical Management of Patients Undergoing Radiation Therapy (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 - Clinical Medicine; 013900 - Radioactivity

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 07	NA	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	.025	0
	CURRENT 74	0	0

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Letterman Army Institute of Research

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) radiation; (U) ionizing radiation; (U) radiation treatment; (U) radiation lethality

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To determine the prognostic value of the concentrations and patterns of serum triglycerides from animals which survive or succumb to x-irradiation. Find agents which alter these patterns to be used both for enchancing effectiveness of radiation therapy or modifying the undesirable side effects of such therapy.

24. (U) Physiological and biochemical manifestations in laboratory animals systematically correlated with the observed clinical course following irradiation. Results evaluated for clues to the development of improved and new methods for diagnosis and treatment of acute and chronic radiation injury. Of particular interest are potential applications of dietary, chemical, and antibiotic therapy of radiation casualties and of radiation-treated patients.

25. (U) 72 07 - 73 06 In vitro tests of membrane's stability were performed to determine whether the anemia following x-irradiation was caused by alterations in erythrocyte membranes. Erythrocytes from sham or x-irradiated mice, pretreated with either SKF-525A or saline, were subjected to concentrations of sodium chloride ranging from .06 to .12 M, to measure the osmotic stability of the membranes, or to 1.0% hydrogen peroxide to measure the membrane's resistance to peroxidation. The erythrocyte's resistance to osmotic pressure or to peroxidation was not significantly affected by either drug treatment or x-irradiation. These results indicate that the anemia following x-irradiation is most likely not attributable to changes in membrane stability and probably reflect changes in the rate of cell synthesis.

*Available to contractors upon originator's approval.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6916	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	K. Complete	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71P	01	092
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) In-vitro Interactions of Digitoxin-I-125 and its Metabolites with Cholestyramine and Tetraethylenepentamine (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 09	NA	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	.5	60.0
	CURRENT 74	0	0

19. RESPONSIBLE DOD ORGANIZATION
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20. PERFORMING ORGANIZATION
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Presidio of San Francisco, CA 94129

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code) (U) beagle
(U) digitoxin; (U) adsorption; (U) cholestyramine; (U) tetraethylenepentamine;

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) 1) To verify the significant adsorption and/or binding penomena of digitoxin by steroid-binding resins in vitro in order to more quickly and effectively reduce the levels in patients treated with digitoxin. 2) Produce metabolites of digitoxin in the beagle in order to study the role of these metabolites play in digitoxin intoxication and the manner in which they may be removed from the blood stream by resin adsorption.

24. (U) Laboratory studies evaluating 1) in vitro intractions of digitoxin and cholestyramine and tetraethylenepentamine.

25. (U) 72 07 - 73 06 Data obtained in this investigation indicates that both cholestyramine and colestipol produce physiologically significant increases in the regression rates of total serum glycosides at elevated levels in the beagle. The postulated intestinal binding of glucuronide and sulfate conjugates, inhibited enteral absorption of free bases, and induction of increased serum digitoxigenin concentration against remaining body pools is accomplished by each resin with qualitative and quantitative uniqueness. The resultant decrease in total serum glycoside half-life with resin therapy appears to be in support of previous studies predicting interruption of enterohepatic circulation of digitoxin by selected anion exchange resins.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 65 (FOR ARMY USE) ARE OBSOLETE.

ANIMAL RESOURCES DIVISION

ANIMAL RESOURCES DIVISION

The Animal Resources Division (ARD) of LAIR provides laboratory animal service for the support of laboratory research as well as divisional research projects conducted by staff members of Letterman Army Medical Center (LAMC). This laboratory animal service consists of supplying healthy research animals to meet investigative requirements, providing acceptable care and housing for these animals, and providing expert personnel and specialized equipment that makes more effective and efficient use of laboratory animals in research possible. Included under these latter services are operation of an experimental surgical laboratory, an animal quality control and disease diagnostic service, and consultation and participation in the development and execution of research proposals involving the use of animals.

Funds for operating the ARD are included in the administrative budget and are indirectly derived from other research line items, except for clinical research support which is provided to LAMC on a reimbursable basis.

During the period covered by this report, the ARD provided care for a total of 245 dogs, 15 cats, 50 sheep, 81 rabbits, 4 monkeys, 658 guinea pigs, 145 hamsters, 350 mice, and 430 rats.

Services provided by the veterinary pathologist in support of the animal research program included 40 autopsies and 2 surgicals for guinea pigs, 14 autopsies and surgicals for mice, 7 autopsies for monkeys, 3 autopsies and 2 surgicals for cats, and 8 autopsies and 1 surgical for other miscellaneous species. Approximately 20% of this activity was in support of the Clinical Research Program.

Of the total number of animals used, 60 dogs, 6 cats, 13 sheep, 9 rabbits, 4 monkeys, and 25 guinea pigs were used in the following clinical research projects, which are not described elsewhere in this report:

1. "Hemodynamics of Spinal Cord Section in Experimental Animals", a study conducted by members of the Neurosurgical

Service, LAMC, has utilized 23 dogs. The occurrence of pulmonary edema in humans that have suffered acute cervical spinal cord section has provided the need for examining in an experimental animal the hemodynamic changes that follow acute spinal cord section and of large volumes of fluid that are frequently given in such human cases therapeutically. Physiological effects are monitored in dogs whose cervical spinal cord has been surgically interrupted by measuring arterial and venous pressures, cardiac output, electrocardiogram, and both the pulmonary artery and its "wedge" pressure as determined by use of a triple lumen Swan-Gantz catheter. Cord section has been found to cause an immediate increase in pulmonary arterial pressure as well as systemic arterial pressure which is followed by cardiac arrhythmia and a pulmonary venous or wedge pressure that is equal to pulmonary arterial pressure. Cardiac output during this interval, as measured by the dye dilution technique, has dropped to values of less than 1 liter per minute. These two findings suggest that the function of the left heart is momentarily impaired during this period. However, pulmonary wedge pressure and cardiac output levels rally after a short interval, although cardiac output levels remain depressed below normal. A progressive drop in arterial blood pressure then follows that proceeds to shock and death, usually within an hour, unless fluid therapy is given. An increased pulmonary wedge pressure has been found to be an earlier and more reliable indicator of this decline than increased central venous pressure, which is normally used. Administration of fluids such as dextran, blood, and plasma, as well as catecholamines, have extended life, but the maintenance of arterial pressure has required large volumes of fluid with a subsequent large increase in cardiac output and eventual failure. Pulmonary edema has occurred under these circumstances as it does in humans, but its appearance seems directly related to the length of time an animal is maintained on fluids. In fact, non-cord sectioned control survives and animals that are volume loaded in this manner have also experienced a similar degree of pulmonary edema. The mechanism of pulmonary edema and its relationship to acute cervical spinal cord section and volume loading with fluids in dogs remains obscure.

2. "Ultrasonic phako-fragmentation and irrigation of cataracts", is a study conducted by LTC John P. Shock, MC, Ophthalmology Service, LAMC. The study has utilized 6 cats

and 9 rabbits to develop the optimum specifications for cataract removal with a modified ultrasonic dental instrument. Experimentation has included the use in normal animals of this instrument with differing frequencies, changing the sizes and shapes of the operating needles, and the use of several new micro surgical instruments that can be used to compliment its use. The modifications have been evaluated for their possible deleterious effect on corneal epithelium, the ciliary body, vitreous, and retina. The instrument has been used successfully in both humans and dogs and has significantly facilitated the removal of advanced senile cataracts. The use of animals has also been a valuable teaching asset for training ophthalmologists in the use of this instrument.

3. "Optic nerve fistulization for chronic papilledema", is a study conducted by MAJ Lewis Lauring, MC, also of the LAMC Ophthalmology Service. Rhesus monkeys are being used in this study to determine if the optic nerve sheath can be surgically decompressed by fistulization to relieve papilledema and possibly restore vision. The study has two major developmental stages in animals. The first is to experimentally create papilledema in large rhesus monkeys, whose orbital structures closely resemble those of humans, and the second is to achieve and evaluate the effects of optic nerve fistulization. To date the study has not progressed beyong the first stage since difficulty has been encountered in experimentally producing papilledema. A silastic balloon has been implanted in the subdural space of a monkey over a cerebral hemisphere and small amounts of radioopaque oil have been injected at frequent intervals into it through a reservoir implanted subcutaneously on the head. Unfortunately, the injected material has leaded from the balloon and is reabsorbed without producing the effect of an expanding space-occupying lesion. Silicone medical fluid has recently been obtained from the Dow Chemical Company which is not reabsorbed and offers the promise of being more successful in causing increased cerebrospinal fluid pressure and papilledema.

4. Glycerol given intravenously as an agent for reduction of intracranial and intraocular pressure is being studied by MAJ Tracy Newkirk, MC, of the Neurology Outpatient Service, LAMC. Although glycerol given orally is effective for this purpose, the

oral route is not suitable for pre-operative administration or for those patients who cannot tolerate fluids given by mouth. Dr. Newkirk's study is designed to show that glycerol can be given intravenously with safety and that it is superior to other intravenous agents that are currently being used. Initial work showed that glycerol given intravenously neither passed from the blood into the brain tissue nor was it toxic; bolus injection of 40-50% glycerol solution in saline were more effective in increasing serum osmolality than lesser concentrations given continously. Thirteen sheep in which hydroscopic psyllium seeds had been implanted intracranially were then used as a model for comparing intravenous glycerol with mannitol for the treatment of intracranial hypertension. It was found that glycerol would produce a more rapid reduction in pressure which lasted longer, and which was accompanied by a much smaller diuresis than was true when mannitol was used. Companion studies in guinea pigs utilizing carbon 14 labeled glycerol injected into a cerebral hemisphere showed that glycerol is rapidly metabolized by the brain. This characteristic provides a unique safety factor since other agents in use are not metabolized and could accumulate in the injured brain to cause a "rebound" increase in intracranial pressure. Also, glycerol may act as an energy source in this instance and assist in the recovery of a partially devitalized brain. Hopefully this work will serve as the introduction of intravenous glycerol in clinical trials for the reduction of intracranial pressure. Such trials are in the early planning stages now.

5. The development of stomach ulcers in patients following the use of continuous suction with a pump type naso-gastric tube has led to a study by CPT John Greene, MC, a pathology resident at LAMC, to compare the effects of continuous and intermittent suction on the gastric mucosa of dogs. In a series utilizing 12 dogs, it was possible to show that ulcers very similar to those that have been observed in four fatal human cases occur in dogs as well following gastric intubation and the application of either continuous or intermittent suction. The dog therefore appears to be a good model for the development of techniques and equipment that could alleviate or eliminate this undesirable effect in cases where the continual removal of stomach fluids is considered necessary.

6. A study is being conducted by CPT Michael Wiener, MC,

a pediatric resident, LAMC, and LTC Robert Kovatch, VC, LAIR, to determine if a vitamin A deficient diet given to pregnant guinea pigs can cause neonatal pulmonary disease resembling the Mikety-Wilson syndrome in guinea pig offspring. To date 25 pregnant guinea pigs have been used in the study, but their extreme reluctance to consume a diet free of vitamin A has resulted in their premature death or abortion and still births of their litters. Accordingly it has not been possible to observe the possible effects of vitamin A deficiency in newborn offspring.

7. LTC John H. Hutton, MC, LAMC, Department of Surgery is examining the suitability of bovine heterografts placed in the dog as a means of avoiding the damage that occurs when needles must be repeatedly placed in blood vessels during dialysis procedures. Despeciated sections of the bovine carotid artery have been placed in a shunt between the femoral artery and vein in three dogs. Although long-term evaluation of the heterograft in the first dog was curtailed because crimping of the graft between the two vessels occurred, improvements in technique in the second and third dogs made extended use of the graft possible. Repeated placement of 14 gauge needles into the grafts has been possible over long periods of time without complications indicating that such a procedure can have beneficial clinical application.

8. Animals are provided for use by the staff of LAMC for training and familiarization with surgical techniques and specialized or new medical equipment. Open heart surgery and training in the use of the heart-lung perfusion machine by thoracic surgeons and their operating room technicians have utilized 12 dogs for this purpose during the report period.

9. The potential toxicity of boron is being examined in experimental animals by MAJ Kenneth Stein, MC, of the LAMC Dermatology Service following his observation that a patient experienced total body alopecia after the chronic ingestion of borate-containing mouthwashes. Colored guina pigs are being given boric acid ad libidum in the drinking water but will not ingest the solutions of greater than 5% which are considered to be necessary for producing signs of intoxication. Other experimental animals and routes of administration are currently being considered.

10. A new dissociative anesthetic, CI744, which is a combination of the phencyclidine derivative tiletamine hydrochloride and the tranquilizer flupyrozapon is being evaluated in animals by CPT George Ward, VC, LAIR ARD. CI744 has been given to 70 sheep and 20 dogs without complication when given in recommended doses. One sheep died, probably from drug overdose, when 10 times the normal dose was inadvertently administered. Hemodynamic studies in dogs have shown the drug produced no significant effects; cardiac output, blood pressure, and EKG remain normal as do pO_2, pCO_2, and pH values in blood gas analysis. The chief advantages of CI744 are that it can be given intramuscularly with ease, it is fast acting (within several minutes), and it produces a cataleptoid type of anesthesia that is intermediate in length without disturbing physiological effects. With these characteristics, this anesthetic shows promise of being very useful for clinical application in military working dogs.

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AKERS, W.A.: "Therapeutic Developments from Military Dermatology" Presented at University of California School of Medicine, San Francisco, CA, March 1973.

CUTCHER, J.L.: "Prevention of Post-Extraction Alveolitis" Presented at the International Association for Dental Research meeting, Washington, DC, April 1973.

HOURIGAN, M.: "Prevention of Post-Extraction Alveolitis" Presented at the American Society of Oral Surgeons, Houston, Texas, September 1972.

HOURIGAN, M.: "Survey or Oral and Maxillofacial Injuries in the Federal Dental Services" Presented at the American Society of Oral Surgeons, Houston, Texas, September 1972.

JOHNSEN, D.O.: "Laboratory Animal Care in Thailand" Presented at the Northern California Branch of the American Association for Laboratory Animal Science, University of California Medical Center, San Francisco, CA, December 1972.

NEVILLE, J.R.: "Hemoglobin Oxygen Affinity Measurement Using Biotonometry" Presented at the Western Pharmacological Society, Squaw Valley, CA, January 1973.

PAYNE, T.F.: "Evaluation of Actinic Blocking Agents for Protection of Lip Mucosa" Presented at the International Association for Dental Research meeting, Washington, DC, April 1973.

ORAL PRESENTATIONS - CONUS FY 1973 (Cont'd)

ROBERT, R.C.: "A Physical Method for Comparing Strength of Bone Graft Union" Presented at the International Association for Dental Research meeting, Washington, DC, April 1973.

SLONE, T.: "The Junior Animal Technician" Presented at the Association of Laboratory Animal Science meeting, San Francisco, CA, May 1973.

ORAL PRESENTATIONS - OCONUS FY 73

TEMPEL, T.R: 'Host Factors in Periodontal Disease: Manifestations Chediak-Higashi Syndrome" Presented at Second International Conference on Periodontal Research, August 1972, Aarhaus, Denmark.

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